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**Drug design and novel anti-cancer therapeutics: inhibitors of 17 [beta] hydroxysteroid dehydrogenase type 3**

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# **Drug Design and Novel Anti-Cancer Therapeutics: Inhibitors of 17 $\beta$ Hydroxysteroid Dehydrogenase Type 3**

**Helen Victoria Bailey**

Volume 1 of 1

A thesis submitted for the degree of Doctor of Philosophy  
University of Bath, Department of Pharmacy and Pharmacology

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## Abstract

Herein, we describe the design and synthesis of novel inhibitors of 17 $\beta$ -hydroxysteroid dehydrogenase type 3 which converts androstenedione into testosterone, which is then converted into dihydrotestosterone (DHT). This isozyme has been implicated in the growth of prostate cancer, which is stimulated by the presence of DHT.

Using an *in silico* pharmacophore model based upon established activity, initial targets were planned, based around a diphenylether hydrophobic head linked to a 4-substituted piperidine ring. Over 45 compounds were synthesised and many show significant biological activity when evaluated in a 17 $\beta$ -HSD Type 3 biological assay. The most potent compound in this series is 1-(4-[2-(4-chloro-phenoxy)-phenylamino]-piperidin-1-yl)-ethanone (**101**) with an IC<sub>50</sub> of 700 nM. The amine linked compounds are significantly more active than the amide equivalents.

Synthesis of the amine-linked compounds was problematic and led to the development of a novel and general microwave assisted procedure for the reductive amination of anilines, enabling aromatic amine-linked compounds to be synthesised in excellent yields.

A series of benzylamine linked inhibitors was also prepared. Over 30 analogues were synthesised and several show very promising biological activity. The most active compound is *N*-(2-([2-(4-chloro-phenoxy)-phenylamino]-methyl)-phenyl)-acetamide (**126**), which exhibits an IC<sub>50</sub> of 900 nM. Effects of chirality were also explored. The enantiomers of *N*-(2-(1-[2-(4-chloro-phenoxy)-phenylamino]-but-2-enyl)-phenyl)-acetamide (**172**) were separated by chiral HPLC and X-ray crystallography was used to determine the absolute configuration. These individual enantiomers and many other novel inhibitors are awaiting biological evaluation.

The synthesis of compounds with a benzophenone linked hydrophobic head group led to an unexpected product. X-ray crystallography was used to determine the structure, as a quinoline derivative. This led to optimisation of a novel modification of the Friedländer synthesis of quinolines.

The potent inhibitors synthesised are selective over 17 $\beta$ -HSD Types 1 and 2. One inhibitor (**165**) also shows potentially interesting activity against the leukaemia cell line CCRF-CEM, in the NCI screening, with a GI<sub>50</sub> of 10 nM.

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## **Publications**

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Novel Inhibitors of 17 $\beta$ -Hydroxysteroid Dehydrogenase Type 3, Nigel Vicker, Joanna M. Day, Helen V. Bailey, Wesley Heaton, Ana M. Ramos-Gonzalez, Christopher M. Sharland, Michael J. Reed, Atul Purohit, Barry V L Potter. WO/2007/003934, 2007.

Novel Inhibitors of 17 $\beta$ -Hydroxysteroid Dehydrogenase Type 3, Nigel Vicker, Joanna M. Day, Helen V. Bailey, Wesley Heaton, Michael J. Reed, Atul Purohit, Barry V L Potter. In preparation.



## Abbreviations

17 $\beta$ -HSD	17 $\beta$ Hydroxysteroid dehydrogenase
Ac	Acetyl
ACN	Acetonitrile
AcOH	Acetic Acid
AKR	Aldo-keto reductase
Ar	Aryl
AR	Androgen Receptor
BID	Dosed twice daily
BINAP	( $\pm$ )-2,2'-Bis(diphenylphosphino)-1,1'-binaphthalene
BMS	Bristol Myers Squibb
Bn	Benzyl
br	Broad (spectral)
CNS	Central nervous system
d	Doublet (spectral)
DCE	1,2-Dichloroethane
DCM	Dichloromethane
	Dissociation Enhanced Lanthanide Fluorescence
DELFI <sup>®</sup>	Immunoassay
DHEA	Dehydroepiandrosterone
DHEA-S	Dehydroepiandrosterone sulphate
DHT	Dihydrotestosterone
DMA	Dimethylacetamide
DMAP	Dimethylaminopyridine
DMF	<i>N, N</i> -Dimethylformamide
DMSO	Dimethyl sulfoxide
DPP	Diphenylphosphate
EBNA	Epstein-Barr virus nuclear antigen
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
eq.	Equivalent
ER	Endoplasmic reticulum
Et	Ethyl
EtOAc	Ethyl acetate
EtOH	Ethanol
FBS	Foetal Bovine Serum
G418	Geneticin <sup>®</sup> an aminoglycoside antibiotic
GI <sub>50</sub>	Concentration causing 50% growth inhibition
HDBC	Hormone Dependent Breast Cancer
HDPC	Hormone Dependent Prostate Cancer
HOBt	1-Hydroxybenzotriazole
HSP	Heat Shock Protein

HRMS	High Resolution Mass Spectrometry
Hz	Hertz
i.p.	Intraperitoneally
IC <sub>50</sub>	Concentration causing 50% inhibition
IR	Infrared
<i>J</i>	Coupling constant (spectral)
Kg	Kilogram(s)
Ki	Inhibition constant
LC <sub>50</sub>	Lethal Concentration (Conc. required to cause 50% cell death)
LCMS	Liquid Chromatography Mass Spectrometry
LH	Luteinizing hormone
LHRH	Luteinizing hormone releasing hormone
lit.	Literature
Lys	Lysine
m	milli, multiplet (spectal)
M	Moles per litre, molecular ion (mass spectrometry)
Me	Methyl
MeOH	Methanol
MHz	MegaHertz
MS	Mass Spectrometry
n	Nano
NADP(H)	Nicotinamide adenine dinucleotide phosphate (reduced)
nd	Not determined
NEAA	Non-essential amino acid
NMR	Nuclear Magnetic Resonance
PMS	Phenazine methosulphate
p.o	Oral dosing
ppm	Parts per million
<i>p</i> -TSA	<i>p</i> -Toluenesulphonic acid
q	Quartet (spectral)
QSAR	Quantitative Structure Activity Relationship
s	Singlet (spectral)
SAR	Structure Activity Relationship
SDR	Short-chain dehydrogenase /reductase
Ser	Serine
SPA	Scintillation Proximity Assay
t	Triplet (spectral)
T	Testosterone
<i>t</i> Boc	<i>tert</i> -butyloxycarbonyl
<i>t</i> Bu	<i>tert</i> -butyl
TEA	Triethylamine
TFA	Trifluoroacetic Acid

TGI	Total Growth Inhibition
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
Tyr	Tyrosine
$\delta$	Delta (chemical shift)

**To Rob and all the Baileys,  
Mandersons and Smiths**

## **Chapter 1:**

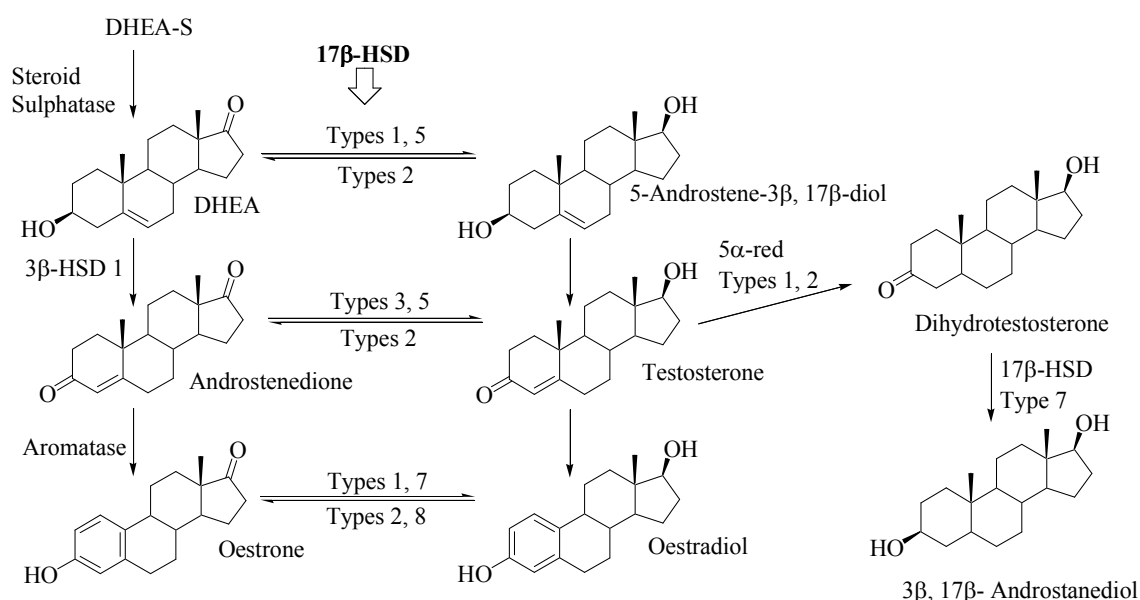
### **Introduction**

#### **1.1 Hormone-Dependent Cancers**

Since the mid-nineteenth century, the average life expectancy in the UK has almost doubled. Whilst this demonstrates positive medical advances and improvement in overall quality of life, it also means that more people are susceptible to cancer. Indeed, cancer is a disease that affects mainly older people, with 64 % of cases occurring in those aged 65 and over.<sup>1</sup> A further staggering statistic is that one in three people will be diagnosed with cancer during their lifetime. In 2004, more than 280,000 new cases of cancer were diagnosed in the UK alone.<sup>1</sup> There are over 200 different types of cancer but the four most common, lung, prostate, breast and large bowel (colorectal) account for over half of all cases diagnosed.<sup>1</sup> Of all human cancers, 40 % are steroid hormone sensitive, (namely breast, prostate, ovarian and uterine cancers).<sup>1</sup> The growth of breast tumours in postmenopausal women is often directly linked to the presence of oestradiol and so these tumours are classified as hormone-dependent breast cancers (HDBC). Hormone-dependent prostate cancer growth, on the other hand, is stimulated by the presence of androgens, specifically dihydrotestosterone (DHT) which is synthesised from testosterone (T).

#### **1.2 Biosynthesis of Androgens in Adult Males**

The biosynthesis of androgens is controlled in the central nervous system (CNS) where luteinising hormone releasing hormone (LHRH) acts on the pituitary to increase luteinising hormone (LH) production. In turn, luteinizing hormone acts on the testes which causes an increase in androgen synthesis.<sup>2</sup> Androgens are synthesised from the inactive adrenal precursors; dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEA-S). DHEA-S levels in adult males are 100 - 500 times higher than the levels of testosterone, thus providing a large reservoir of substrate for conversion into androgens. The rate of formation of androgens is therefore dependent upon the level of expression of enzymes involved in the biosynthesis of active androgens (Scheme 1.1).<sup>3</sup> Once synthesised these hormones can then act upon the appropriate receptors.



**Scheme 1.1:** Steroidogenic Pathway.<sup>4</sup>

Active steroids are removed from circulation by metabolism in peripheral tissues to inactive molecules and subsequently into water soluble molecules for excretion from the body. This is achieved by the formation of the sulphate or glucuronide derivatives on the hydroxyl groups of the steroids. UDP-glucosyltransferases inactivate testosterone, DHT and androsterone to their respective glucuronides.<sup>5</sup>

### 1.3 The Androgen Receptor

The androgen receptor (AR) is a member of the steroid and nuclear receptor superfamily, which has over 100 members.<sup>6</sup> However, only 5 vertebrate steroid receptors (oestrogen, progesterone, androgen, glucocorticoid and mineralocorticoid) are known. The AR is a soluble protein that functions as an intracellular transcription factor.<sup>6</sup> It is mainly found in androgen target tissues, such as prostate tissue, skeletal tissue, muscle tissue, liver tissue and tissue in the central nervous system (CNS).<sup>6</sup> The AR gene has been localised to the human X chromosome and to date only one AR gene has been identified. This AR gene is more than 90 kb long, and codes for a protein of 919 amino acids, which has three main domains; the *N*-terminal domain, the DNA-binding domain and the ligand-binding domain. The DNA binding domain is highly conserved between the vertebrate steroid receptors (sequence similarity 59-82 %) reflecting the common need to bind to DNA. Sequence identity in the ligand-binding domain is lower (ranging from 22 to 55 %) showing the receptor selectivity for specific hormones.<sup>6</sup> The crystal structures of the DNA-binding domain and the ligand-binding domain of the AR have been solved, but as yet, no crystal structure of the full receptor is available.

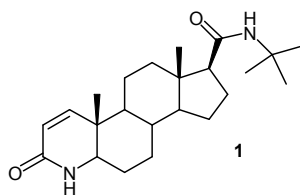
Unbound AR is found mainly in the cytoplasm and is associated with a complex of heat shock proteins (HSPs).<sup>6</sup> Upon androgen binding, the AR goes through a series of conformational changes. Firstly, the HSPs dissociate and the AR undergoes dimerisation, phosphorylation and translocation to the nucleus.<sup>6</sup> The receptor can then bind to the androgen response element and initiate the recruitment of transcription co-regulators which further ensure the amplification of AR regulated gene expression. This is the genomic pathway, but the AR can also be activated via a non-genomic pathway, either by rapid activation of kinase signalling cascades or modulation of intracellular calcium levels. This non-genomic pathway of the AR has been reported in skeletal muscle cells, osteoblasts and prostate cancer cells.<sup>6</sup> It has been proposed that specific ligands may cause the separation of genomic and non-genomic pathways and this might help to achieve tissue specificity. However, the essential structural features for achieving this separation have not been determined.<sup>6</sup>

#### **1.4 Prostate Cancer**

Prostate cancer is the second most common cancer in males in western countries with 679,000 new cases diagnosed each year (based upon 2002 data).<sup>7</sup> In fact, it causes over ~200,000 deaths per year worldwide.<sup>7</sup> This represents a therapeutic area with a great unmet medical need. Prostate cancer is often a very slow growing disease and watchful waiting is often the first stage of medical intervention. If it is deemed that the prostate cancer is growing at a significant rate to affect the patient in terms of quality or longevity of life then further treatments are initiated. Currently, a surgical procedure, radical prostatectomy, is one of the main treatments for prostate cancers. This involves the removal of the prostate gland, seminal vesicles and nearby lymph nodes. This is a major operation and therefore has associated risks. The possible side effects include urinary incontinence, sterility and erectile dysfunction. Radiotherapy is an alternative treatment, in which radiation is applied to the affected area to destroy the cancer cells. This can also cause undesirable side effects, including bladder irritation, erectile dysfunction and diarrhoea.<sup>8</sup>

Androgens are necessary for the initiation of prostate cancer, the balance between androgen induced cell proliferation and apoptosis regulating growth. A change in this balance can lead to excessive androgen influence and increased cell proliferation. This dependency has been exploited for the treatment of prostate cancer since 1941.<sup>5</sup> There are several methods of androgen deprivation in the clinical treatment of prostate cancer. Limitating the activity of androgens on prostate tissue reduces both cancerous and non-cancerous prostate growth. Castration is the frontline treatment, defined by a reduction in serum testosterone levels to <50 ng/ mL and this can be achieved surgically or

medically. Surgical castration results in a >90% decrease in serum testosterone, but only a 50% decrease in levels in the prostate, indicating that there is a significant local source of hormone synthesis.<sup>2</sup> This will be discussed in detail later. Medical castration can be achieved with 5 $\alpha$ -steroid reductase inhibitors, gonadotropin-releasing hormone (GnRH) agonists, GnRH antagonists or oestrogen agonists.<sup>9</sup> For example, finasteride (Figure 1.1, Proscar™, **1**) limits DHT production by inhibiting the enzyme, 5 $\alpha$ -steroid reductase type 2, which is responsible for the conversion of T into DHT. This reduces DHT to 20-35% of baseline levels, thus helping to reduce the size of the prostate, often before surgery or radiation therapy. Finasteride is a very potent inhibitor of 5 $\alpha$ -steroid reductase with an IC<sub>50</sub> value of 9.4 nM.<sup>10</sup> It does not however completely eradicate DHT.



**Figure 1.1:** Finasteride (Proscar™).

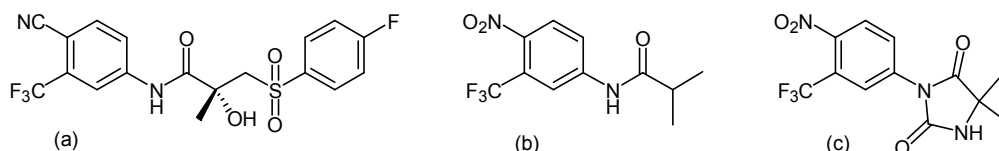
GnRH agonists and antagonists can also be used to achieve medical castration. GnRH is a small hormone, composed of 10 amino acids, which is secreted directly into the portal blood circulation.<sup>9</sup> It interacts with high-affinity receptors in the anterior pituitary, stimulating the release of LH, which consequently leads to an increase in androgen production. Continuous stimulation with high concentrations of GnRH results in receptor desensitisation and a reduction in LH release leading to a lower level of testosterone production in the testes.<sup>9</sup> Endogenous GnRH has a very short half life due to peptidase degradation. However, synthetically produced analogues, including goserelin, leuprorelin, buserelin and triptorelin, have been developed which have an increased resistance to peptidase degradation and therefore have a much longer half life.<sup>9</sup> These are administered by subcutaneous injection every 1-3 months and ~ 95% of patients achieve significant testosterone suppression. However, the initial increase in LH production, causing a corresponding increase in T and DHT levels, causes a tumour flare effect and, therefore, combination therapy with an anti-androgen is normally used for the first few weeks.<sup>9</sup> GnRH antagonists can also be used as these cause a major and rapid reduction in serum T levels. They also have the advantage that the tumour flare effect seen with GnRH agonists is avoided, and the more rapid decrease in serum T levels may increase the success of treatment.<sup>9</sup>

Unfortunately, castration, whether medical or surgical, can cause a wide variety of detrimental side effects, known as the castration syndrome. These side effects can include: loss of libido, erectile dysfunction, hot flushes, anaemia, obesity, fatigue, a



decrease in muscle strength, a decline in physical activity and vitality, mood changes, depression and osteoporosis. These all affect the quality of life of the patient and, therefore, must be taken into account when considering castrative treatment for patients.<sup>9</sup>

A treatment which avoids castration is the use of anti-androgens, such as bicalutamide (Casodex<sup>TM</sup>), flutamide (Eulexin<sup>TM</sup>) and nilutamide (Nilandron<sup>TM</sup>). These compounds are all orally administered non-steroidal compounds (Figure 1.2), which inhibit the activity of androgens by competitively blocking the interaction of T and DHT with the androgen receptor. They all offer many quality of life benefits in terms of sexual interest, physical capacity and preservation of bone mineral density.<sup>9</sup> Bicalutamide (Casodex<sup>TM</sup>) was launched by AstraZeneca in 1995, as a combination therapy, with medical or surgical castration, for advanced stage prostate cancer.<sup>11</sup> It has replaced flutamide and nilutamide as the anti-androgen of choice for prostate cancer treatment, as it has less hepatotoxicity and a longer half-life (6 days), which allows for once daily dosage.<sup>6</sup> It was later licensed as a monotherapy for early stages of the disease.<sup>11</sup> However, in 2003 Casodex<sup>TM</sup> (150 mg) was withdrawn, after research showed an increase in mortality for Casodex<sup>TM</sup> versus placebo.<sup>12</sup> Anti-androgens can also often trigger significant increases in LH release, which causes an increase in serum T levels.<sup>6</sup> Figure 1.4 shows the crystal structure of the ligand binding domain of the androgen receptor in complex with *R*-bicalutamide.<sup>13</sup>



**Figure 1.2:** (a) Bicalutamide (Casodex<sup>TM</sup>), (b) flutamide (Eulexin<sup>TM</sup>), (c) nilutamide (Nilandron<sup>TM</sup>).

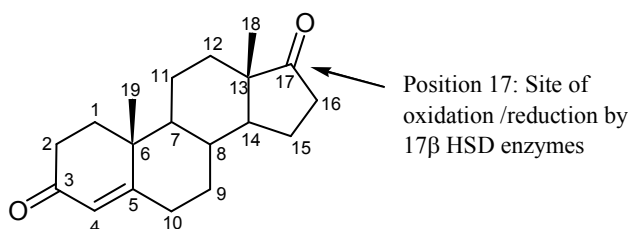
The widespread use of prostate-specific antigen (PSA) as a detection marker for prostate cancer has led to earlier diagnosis and detection of the disease. Although this represents a major advance in prostate cancer diagnosis and treatment, it has caused a dramatic shift in the population of patients requiring treatment, as more cases are diagnosed in younger males. Therefore, the systematic side effects of androgen deprivation and the effects on the quality of life have become more important. At present, the strategies improve survival, but are not generally curative and also may lower the overall quality of life of the patient. The main problem is that prostate cancer often advances to a “hormone refractory” state, in which the disease no longer responds to treatment with anti-androgens, or to castration therapies. There is evidence to suggest that the AR is at the centre of this process.<sup>14</sup> It has been reported that a 2 to 5 fold increase in the AR

mRNA is both necessary and sufficient for progression to the “hormone refractory” disease state.<sup>15</sup> In the presence of elevated levels of AR in hormone refractory cancers, cells become highly sensitive to androgens. This evidence provides a strong rationale for targeting the down regulation of AR to treat advanced prostate cancer. Reducing the AR to a critical level would slow the growth and proliferation of prostate cancer. Several approaches are currently available, including small molecule approaches,<sup>16-18</sup> HSP-90 inhibitors<sup>19</sup> and RNA interference.

### 1.5 The 17 $\beta$ -Hydroxysteroid Dehydrogenase Enzymes

The androgen biosynthetic pathway is continuing to be an interesting and challenging area of research for the discovery of new potential treatments for hormone dependent prostate cancer. There are many different enzymes involved in the synthetic pathways from DHEA to T and DHT (Scheme 1.1). These enzymes include: steroid sulphatase, 3 $\beta$ -hydroxysteroid dehydrogenase (types 1 and 2), 5 $\alpha$ -steroid reductase (types 1 and 2) and several members of the 17 $\beta$ -hydroxysteroid dehydrogenase family (Scheme 1.1).<sup>3</sup>

The 17 $\beta$ -hydroxysteroid dehydrogenase enzymes (17 $\beta$ -HSDs, EC1.1.1.62, Table 1.1) catalyse the oxidoreduction of the hydroxyl/carbonyl groups on position 17 (see Figure 1.3) of androgens and oestrogens (using NAD(P)(H) as a co-factor). The 17 $\beta$ -HSDs therefore provide a pre-receptor control mechanism as 17 $\beta$ -hydroxylated forms have a significantly higher binding affinity with the appropriate receptors than the 17 $\beta$ -carbonyl steroids, causing transactivation of the target genes.<sup>4</sup>



**Figure 1.3:** Androstenedione and the site of action of the 17 $\beta$ -HSD enzymes.

Numbered in the order of discovery, there are, at present, 14 members of the 17 $\beta$ -HSD subfamily identified (Table 1.1), although the 17 $\beta$ -HSD type 13 isozyme has only recently been reported.<sup>20†</sup> It is known that 12 of these isozymes exist in humans. Those isozymes not found in humans are types 6 and 9 and they have been identified in rats and mice respectively.<sup>21</sup> Six of the isozymes have been structurally characterised, namely, 1, 4, 5, 10, 11 and 14. Although most of the isozymes are capable of catalysing

<sup>†</sup> The 17 $\beta$ -HSD type 13 isozyme was disclosed at a workshop on 11 $\beta$ - and 17 $\beta$ -HSDs: Role in Human Disease, Elmau Castle, Germany, 2005, and identified and previously classified as human retinal shortchain dehydrogenase/reductase 3, PDB code 1YDE.

both the oxidative and reductive reactions *in vitro*, *in vivo* they have preferred directionality and can therefore be classified into two categories: a) oxidative enzymes (17 $\beta$ -HSD types 2, 4, 6, 8, 9, 10, 11, 13 and 14), which catalyse the NAD(P)<sup>+</sup>-dependent oxidation *in vivo* and therefore the inactivation of steroids, and b) reductive enzymes (17 $\beta$ -HSD types 1, 3, 5 and 7), which catalyze the NAD(P)H dependent reduction of ligands *in vivo* forming active steroid hormones.<sup>20</sup> All but one of the isozymes are members of the short-chain dehydrogenase/reductase (SDR) family. The exception is 17 $\beta$ -HSD type 5, which is an aldo-keto reductase (AKR).<sup>3</sup> SDR enzymes constitute a large family of oxidoreductases. To date 63 SDR members have been identified within the human genome.<sup>22</sup> They are mainly oligomeric enzymes, with a typical chain length of 250-350 amino acid residues. They share few distinct sequence motifs and contain a typical sequence identity of 15-30%. However, SDR enzymes have a conserved  $\alpha/\beta$  sandwich folding pattern (Rossmann-fold) and a highly conserved Tyr-X-X-X-Lys sequence in the active site, as a general acid/base catalyst. This is facilitated by the lowering of the Tyr-OH pKa through a neighbouring lysine residue, and substrate carbonyl binding is aided through a conserved serine side chain.<sup>20</sup> The SDR enzymes are mostly NAD(P)(H) dependent and act on a wide substrate spectrum, including steroids, retinols, prostaglandins, polyols and xenobiotics.

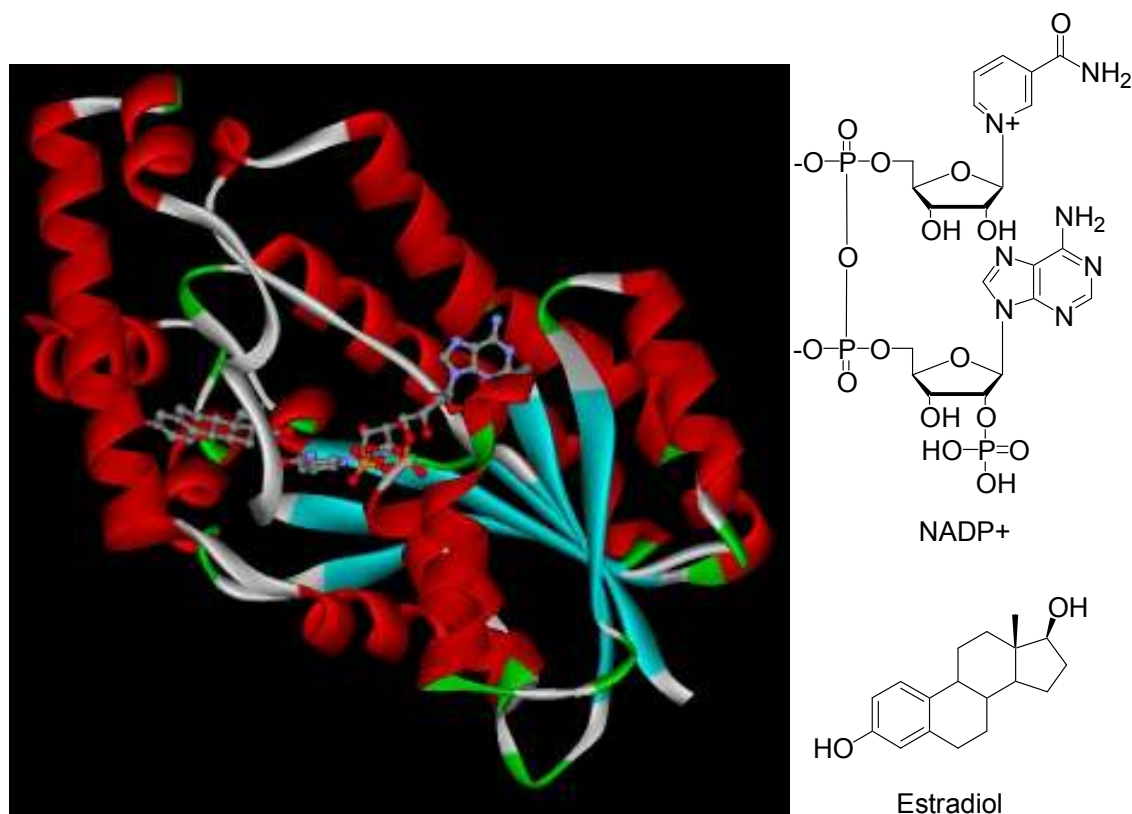
High levels of 17 $\beta$ -HSD expression have been measured in prostate cancer cell lines, indicating that some of the 17 $\beta$ -HSDs are thought to be significant to hormone dependent prostate cancer. Others are implicated in hormone-dependent breast cancer. The enzymes of interest are 17 $\beta$ -HSD types 1, 2, 3 and 5. These therefore represent potential drug targets and will be discussed further.

Human Protein	Type	Function	PDB code	Cofactor preference	Substrates	Subcellular localization	Expression pattern	Disease or Pathology
<b>17<math>\beta</math>HSD1</b>	SDR	E2 production	1FDT, 1EQU, 1JTV	NADP(H)	oestrogens	Soluble in cytosol	widespread; gonads, breast, placenta, liver	Breast and prostate cancer
<b>17<math>\beta</math>HSD2</b>	SDR	E2, T inactivation		NAD(H)	oestrogens, androgens, progestins	Membrane bound on ER	widespread; prostate, liver, intestine	Endometriosis, colon and prostate cancer,
<b>17<math>\beta</math>HSD3</b>	SDR	T production		NADP(H)	androgens	Membrane bound on ER	mainly testis	Pseudohermaphroditism and prostate cancer
<b>17<math>\beta</math>HSD4</b>	SDR	$\beta$ -oxidation of FA, E2 inactivation	1ZBQ	NAD(H)	oestrogens, acyl CoAs	peroxisomes	widespread	D-specific multifunctional protein deficiency, Stiff-man syndrome
<b>17<math>\beta</math>HSD5</b>	AKR	T production, 20 $\alpha$ P activation, bile acid production and detoxification,	1ZQ5 2FGB	NADP(H)	androgens, eicosanoids	Soluble in cytosol	liver, prostate	
<b>17<math>\beta</math>HSD7</b>	SDR	Cholesterol synthesis and E2 production		NADP(H)	oestrogens, cholesterol	Membrane bound on ER		
<b>17<math>\beta</math>HSD8</b>	SDR	E2 and androgen inactivation		NAD(H)	androgens		widespread, liver, kidney	
<b>17<math>\beta</math>HSD10</b>	SDR	Estrogen and androgen inactivation, $\beta$ -oxidation of FA, bile acid isomerisation	1SO8 1UZT	NAD(H)	oestrogens, androgens, bile acids, progestions, branched/straight OH-acyl CoAs	mitochondria	widespread, liver, CNS, kidney, testis	Isoleucine degradation deficiency, Alzheimer disease
<b>17<math>\beta</math>HSD11</b>	SDR	Conversion of 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol to androsterone	1YB1	NAD(H)	androgens	Membrane bound on ER	steroidogenic tissues	
<b>17<math>\beta</math>HSD12</b>	SDR	3-Ketoacyl-CoA reductase; FA synthesis		NADP(H)	Steroids acyl CoAs			
<b>17<math>\beta</math>HSD13</b>	SDR	Activity not known	1YDE					
<b>17<math>\beta</math>HSD14</b>	SDR	E2, T inactivation; $\beta$ -oxidation		NAD(H)	oestrogens, androgens, fatty acyl CoAs	Soluble in cytosol	CNS, kidney	

**Table 1.1:** Summary of the 14 human isozymes of 17 $\beta$ -HSD.<sup>20, 21</sup>

## 1.6 17 $\beta$ -Hydroxysteroid Dehydrogenase Type 1

The 17 $\beta$ -HSD Type 1 (17 $\beta$ -HSD1) isozyme was the first 17 $\beta$ -HSD to be cloned and structurally characterised. It is a soluble cytosolic protein which consists of 327 amino acids and has a molecular mass of 35 kDa.<sup>23</sup> The protein has been crystallised, and this led to the first elucidation of the three-dimensional structure of a mammalian steroidogenic enzyme. The core of the structure is a seven-stranded, parallel  $\beta$ -sheet, surrounded by 6 parallel  $\alpha$ -helices, three on each side of the  $\beta$ -sheet. The binding site consists of a narrow hydrophobic tunnel, with Tyr155, Lys159 and Ser142 forming the catalytic triad of the enzyme.<sup>24</sup> The crystal structure of 17 $\beta$ -HSD Type 1 complexed with oestradiol and NADP<sup>+</sup> is shown in Figure 1.4.<sup>25</sup>



**Figure 1.4:** The crystal structure of 17 $\beta$ -HSD Type 1 complexed with estradiol and NADP<sup>+</sup> (PDB code 1FDT).<sup>25</sup>

The enzyme preferentially reduces estrone to oestradiol, and also to a more minor extent, reduces DHEA to 5-androstene-3 $\beta$ , 17 $\beta$ -diol.<sup>21</sup> In humans, it is predominantly expressed in the ovaries, breast tissues, placenta and is expressed at a greater level in breast carcinoma.<sup>20</sup> Oestradiol exerts oestrogenic effects by binding with the oestrogen receptor which leads to a proliferative effect which accelerates tumour growth.<sup>10</sup> Therefore, a specific 17 $\beta$ -HSD1

inhibitor would potentially lower the levels of estradiol and the proliferative effects exhibited in hormone dependent breast cancer. Currently, there is a lot of interest in this area of research, with many reversible and irreversible 17 $\beta$ -HSD type 1 inhibitors having been reported.<sup>10</sup> The structures of the inhibitors are very wide ranging. Although most are based around a steroidal backbone, they are often analogues of the natural substrate, estrone (Scheme 1.1).<sup>26, 27</sup> However, non-steroidal compounds have also been reported as inhibitors. For example, fatty acids such as oleic acid have demonstrated moderate inhibitory activity.<sup>28</sup> Research in this area is currently very active and the *in vivo* anti-tumour efficacy of an agent against this target has been demonstrated.<sup>26, 29, 30</sup>

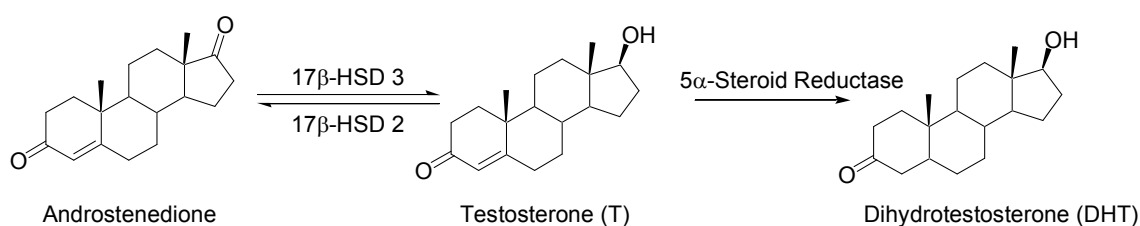
### 1.7 17 $\beta$ -Hydroxysteroid Dehydrogenase Type 2

The 17 $\beta$ -HSD type 2 (17 $\beta$ -HSD2) isozyme catalyses the NAD(H)-dependent conversion between estradiol, testosterone and 5 $\alpha$ -dihydrotestosterone and the corresponding 17-oxo metabolites. Due to its widespread tissue distribution it is thought that 17 $\beta$ -HSD Type 2 acts to protect against damaging levels of active steroid hormone.<sup>20</sup> It was cloned from a human prostate cDNA library and is thought to be localised on the endoplasmic reticulum (ER).<sup>31</sup> However, the highly hydrophobic nature of the enzyme has prevented structure determination thus far. The enzyme is a protein of 387 amino acids and has a molecular mass of ~ 42.8 KDa.<sup>31</sup>

### 1.8 17 $\beta$ -Hydroxysteroid Dehydrogenase Type 3

The 17 $\beta$ -HSD type 3 isozyme is an interesting drug target for limiting the biosynthesis of T and DHT. Targetting this enzyme would lower the amount of active DHT available to stimulate an androgenic response in androgen-dependent diseases such as prostate cancer. 17 $\beta$ -HSD3 is principally found in the Leydig cells of the testes so selective inhibitors may also have the potential to block spermatogenesis and function as a male anti-fertility agent.<sup>28, 32</sup>

The 17 $\beta$ -HSD type 3 isozyme consists of 310 amino acids and has a molecular mass of ~34.5 KDa.<sup>24</sup> It is a microsomal enzyme, which is bound through an *N*-terminal transmembrane domain to the ER.<sup>20</sup> It uses NADP(H) as a co-factor, and the equilibrium favours the reduction of androstenedione to testosterone, which is then converted by 5 $\alpha$ -steroid reductase to the most potent androgen, DHT (Scheme 1.2). This reaction occurs in the Leydig cells of the testis under the control of the pituitary hormones.<sup>5</sup>



**Scheme 1.2:** The role of 17β-HSD Type 3 in androgen synthesis.

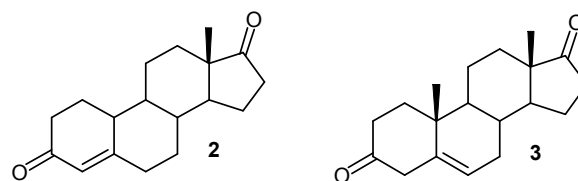
Mutations in the 17β-HSD3 gene can result in the malfunctioning or lack of this enzyme. This leads to an accumulation of androstenedione and also gives rise to an autosomal recessive form of male undermasculinity: male pseudohermaphroditism.<sup>33</sup> This is phenotypically characterised by the presence of testes and normally developed Wolffian duct derivatives. However, patients show undervirilisation of the external genitalia. These are often female in appearance. Therefore, patients are most often raised as females.<sup>34, 35</sup> In contrast 17β-HSD Type 3 deficiency in females is asymptomatic.

### 1.9 17β-Hydroxysteroid Dehydrogenase Type 5

The main biosynthesis of testosterone occurs in the testis, catalysed by 17β-HSD Type 3. However, conversion of androstenedione into testosterone also occurs to some extent in the prostate, this is catalysed by 17β-HSD Type 5. The 17β-HSD type 5 (17β-HSD5) isozyme is highly expressed in the testes and extragonadal tissues such as basal cells of the prostate, adrenal glands and liver.<sup>20</sup> It is the only 17β-HSD enzyme that belongs to the aldo-keto reductase (AKR) superfamily and has a much broader substrate specificity than 17β-HSD types 1-3. It catalyses the NADPH dependent reduction of androstenedione to testosterone, as well as displaying many other activities, such as 3α-HSD, 20α-HSD and prostaglandin synthase activities. Since 17β-HSD Type 3 is not expressed in the ovary, which is the location of androgen synthesis in women, it has therefore been suggested that 17β-HSD Type 5 is responsible for the biosynthesis of testosterone in females as this enzyme is found in the ovary. It has been hypothesised that this enzyme is responsible for the rise in levels of testosterone at puberty in patients with a malfunctioning 17β-HSD3 enzyme (male pseudohermaphroditism).<sup>36</sup> As 17β-HSD Type 5 is directly involved in the biosynthesis of androgens it is an emerging as an interesting therapeutic area for the treatment of prostate cancer, along with 17β-HSD Type 3. The crystal structure has been determined both alone and in complex with different substrates and inhibitors, thus providing an excellent basis for inhibitor development.<sup>20</sup>

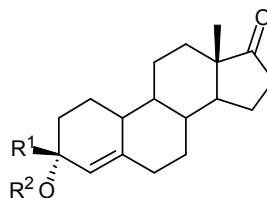
### 1.10 Inhibitors of 17 $\beta$ -HSD Type 3: Steroidal Inhibitors.

The first study on inhibition of 17 $\beta$ -HSD Type 3 was reported in 1983, when Pittaway revealed that a steroid scaffold with a 17-keto group and non-aromatic A-ring were important factors for inhibition.<sup>37</sup> They identified 4-estrene-3, 17-dione (**2**) and 5-androstene-3, 17-dione (**3**) as inhibitors of 17 $\beta$ -HSD Type 3, with  $K_i$  values of 2.4 and 6.8  $\mu$ M respectively (Figure 1.5).



**Figure 1.5:** Early Identified Inhibitors of 17 $\beta$ -HSD3.<sup>37</sup>

Since this initial research, many other steroid inhibitors have been synthesised and evaluated. Recently, Poirer and co-workers published promising work concerning androsterone-3 $\beta$ -substituted derivatives as inhibitors of 17 $\beta$ -HSD3 (Table 1.2).<sup>32, 38</sup>



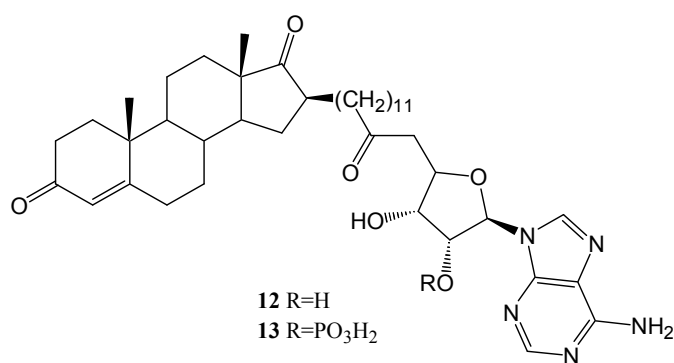
	R <sup>1</sup>	R <sup>2</sup>	Activity over 17 $\beta$ -HSD Type 3 IC <sub>50</sub> (nM)
Androsterone	H	H	330
4	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>2</sub>	H	67
5	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>5</sub>	H	100
6	CH <sub>3</sub> CH <sub>2</sub> (CH <sub>3</sub> )CH	H	73
7	cyclohexyl-CH <sub>2</sub> -CH <sub>2</sub>	H	60
8	phenyl	H	81
9	phenyl-CH <sub>2</sub>	H	57
10	phenyl-CH <sub>2</sub> -CH <sub>2</sub>	H	99
11	phenyl-CH <sub>2</sub> -CH <sub>2</sub>	CH <sub>3</sub>	73

**Table 1.2:** Some of the androsterone derivatives tested for 17 $\beta$ -HSD3 inhibition.<sup>32</sup>



The inhibitors were tested for biological activity using a homogenised cell preparation, made from human embryonic kidney 293 (HEK-293) cells, which had been transfected with vectors encoding for 17 $\beta$ -HSD Type 3. The inhibitors exhibited IC<sub>50</sub> values ranging between 57 and 100 nM.<sup>32</sup> Although they were less active in whole cells, 8 compounds (**4-11**) exhibited >95 % inhibition at 10  $\mu$ M. Selectivity was also demonstrated. The compounds did not show any inhibitory activity on isozymes 1, 2, 5 and only poor inhibition (13-38 %) on isozyme 7, at 10  $\mu$ M, and thus, are selective inhibitors of 17 $\beta$ -HSD Type 3. The binding affinities for androgen, estrogen, glucocorticoid and progesterone receptors were also analysed. None of the above compounds showed any significant binding affinity for these receptors.<sup>32</sup> However, when the compounds were analysed for residual hormonal activity, by looking at proliferative activities on cells expressing the androgen receptor, some activity was observed. Compounds **4, 5, 6, 7, 10** and **11** showed minimal effects, but compounds **8** and **9** showed a 72 % and 82 % enzyme inhibitory activity respectively at 1  $\mu$ M. It can be assumed that there is a different mechanism for this activity as these effects are not achieved through androgen receptor mechanisms. Compounds **5, 7** and **10** emerged as interesting candidates for further investigation.<sup>32, 38</sup>

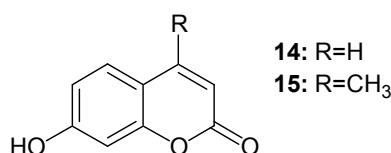
The Poirier research group has also looked at the synthesis and 17 $\beta$ -HSD Type 3 inhibitory activity of androstenedione/adenosine hybrid compounds, (Figure 1.6).<sup>39</sup> These compounds were thought to possess bisubstrate inhibition properties, by blocking both the cofactor binding site and the substrate binding site simultaneously. It was hypothesised that the phosphorylated compound (**13**) would be a better inhibitor than the non-phosphorylated compound (**12**), as the enzyme has a higher affinity for NADPH than NADH and thus that the enzyme would also have a higher affinity for a NADPH mimic than a NADH mimic.<sup>40</sup> However, once the compounds had been tested for 17 $\beta$ -HSD Type 3 activity the results were actually contrary to the hypothesis, with the phosphorylated compound (**13**) proving to be a slightly less potent inhibitor of 17 $\beta$ -HSD Type 3, when compared to the non-phosphorylated compound (**12**), with IC<sub>50</sub> values of 1.66 and 0.62  $\mu$ M respectively. The reasons for this lower activity were investigated and it was suggested that the cofactor NADPH, which is present in a much higher concentration than the inhibitor, would bind first to the enzyme, so that the cofactor binding site would be occupied and thus the potential inhibitor can only interact with the substrate-binding site. This would result in lower activity than if the compounds were true bisubstrate inhibitors.<sup>40</sup>



**Figure 1.6:** Androstenedione/adenosine hybrid compounds as 17 $\beta$ -HSD3 inhibitors.

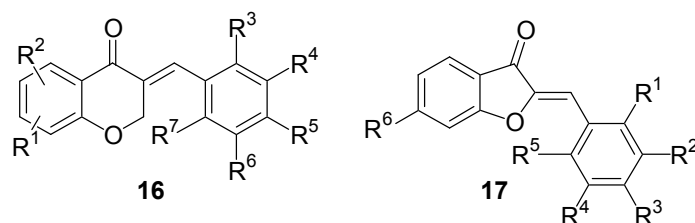
### 1.11 Inhibitors of 17 $\beta$ -HSD Type 3: Non-Steroidal Inhibitors

The steroid based structures of the inhibitors discussed so far may preclude them from further development due to undesirable pharmacological properties, or other undesirable features such as intrinsic androgenic activities. For this reason the design and synthesis of non-steroidal inhibitors has been receiving considerable interest over the past few years.<sup>28</sup> Many different classes of non-steroidal compounds have been identified as inhibitors of 17 $\beta$ -HSD3, these include *p*-benzoquinones, flavones, *iso*-flavones, coumarins and triphenylethene derivatives.<sup>28</sup> However, many of these were shown to be non-selective and/or had various levels of androgenic or oestrogenic activity. The coumarins proved to be an interesting family of compounds, in particular umbelliferone (**14**) and 4-methylumbelliferone (**15**), with IC<sub>50</sub> values of 1.4 and 0.9  $\mu$ M respectively, (Figure 1.7).<sup>41</sup>



**Figure 1.7:** Coumarin inhibitors of 17 $\beta$ -HSD3

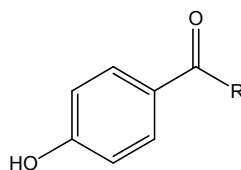
Novel tetralone, benzopyranone and benzofuranone derivatives with general structures **16** and **17**, were the focus of a patent filed in 1998 as preventative and/or curative treatments for various hormone sensitive diseases (Figure 1.8).<sup>42</sup>



Where R<sub>1-7</sub> can include H, Cl, Br, F, Me amongst others

**Figure 1.8:** Novel 17β-HSD3 inhibitors

Lota *et al* recently reported a study where they looked into the possible transition states of the enzyme catalysed reduction reaction in order to design novel inhibitors.<sup>43, 44</sup> They established the importance of a steroid backbone mimic region and a carbonyl moiety in the correct orientation. For this reason a series of 4-hydroxyphenyl ketones were tested for their inhibitory potential, a selection is shown in Table 1.3. From the biological data, obtained from an enzymatic assay (from rat testicular microsomes), it can be seen that the inhibitory activity increases with increasing alkyl chain length, up to a maximum of C<sub>8</sub>. After this point an increase in chain length leads to a reduction in activity.

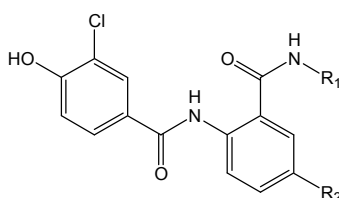


Compound Number	R	IC <sub>50</sub> (μM)
18	CH <sub>3</sub>	1709
19	C <sub>4</sub> H <sub>9</sub>	61
20	C <sub>8</sub> H <sub>17</sub>	2.9
21	C <sub>11</sub> H <sub>23</sub>	7.6

**Table 1.3:** A selection of the inhibitors reported by Lota *et al*<sup>43</sup>

By utilising a molecular modelling study, it was hypothesised that the octyl chain is the optimum length which directly mimics the steroid backbone and the longer chains introduce unfavourable steric interactions in the active site. It is also hypothesised that the 4-hydroxyphenyl moiety is able to undergo hydrogen bonding with the active site. This has been validated by the evaluation of various inhibitors (not reported) which lacked this hydrogen bond forming ability. These compounds were either weak or did not inhibit the enzyme.

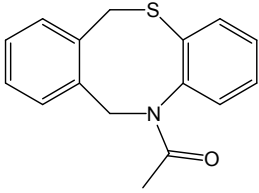
A high-throughput screening programme of >200,000 compounds by Bristol-Myers Squibb revealed that anthranilamide based compounds showed some 17 $\beta$ -HSD Type 3 activity.<sup>2</sup> A limited amount of Structure-Activity Relationship (SAR) data is available (Table 1.4). It can be seen that substitution on the 4-position ( $R_2$ ) of the anthranoyl moiety is very beneficial to activity (compounds **25** vs. **23**) and the introduction of differing groups on the 2-position ( $R_1$ ) can help to improve the activity of the inhibitors. However, it appears that as the enzymatic inhibitory activity increases the cellular potency decreases, perhaps indicating a problem with cell permeability and solubility of the compounds within the series (compounds **22-24**). It is anticipated that this series can be effectively modified to have more favourable drug-like properties.<sup>2</sup>



Compound no	$R_1$	$R_2$	Enzyme IC <sub>50</sub> (nM)	Cellular IC <sub>50</sub> (nM)
<b>22</b>		OPh	60	300
<b>23</b>		OPh	40	710
<b>24</b>		OPh	1	1200
<b>25</b>		H	170	1600

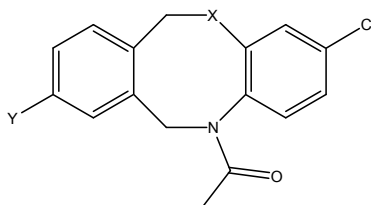
**Table 1.4:** Novel series of anthranilamide based inhibitors.<sup>2</sup>

Bristol-Myers Squibb have also reported a series of tetrahydrodibenzazocines that are potent inhibitors of 17 $\beta$ -HSD Type 3.<sup>45</sup> They also identified these initial lead structures following high-throughput screening using an enzymatic scintillation proximity assay (SPA). Following this, the cellular effects were also examined before the lead compounds were identified. The dibenzothiazocine, compound **26** (Table 1.5), was identified as an attractive starting point for the Bristol-Myers Squibb medicinal chemistry programme. This compound is discussed in more detail in Chapter 2.

	Enzyme IC <sub>50</sub> (μM)	Cellular IC <sub>50</sub> (μM)
 <b>26</b>	0.40	0.32

**Table 1.5:** Compound **26** and the inhibitory activity reported.<sup>45</sup>

Modification of this scaffold led to increases in potency, as shown in Table 1.6. The best compound reported (**31**) had an additional methyl benzoate group. This extra substitution led to a substantial increase in activity. It is hypothesised that this group lies near to the nicotinamide ring within the active site, suggesting a ring-ring interaction, possibly  $\pi$  stacking.

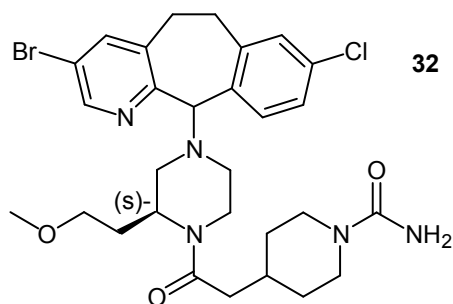


Compound no.	X	Y	Enzyme IC <sub>50</sub> (nM)	Cellular IC <sub>50</sub> (nM)
<b>27</b>	S	H	100	160
<b>28</b>	CH <sub>2</sub>	H	70	63
<b>29</b>	CH <sub>2</sub>	-Ph( <i>o</i> -OMe)	2	5
<b>30</b>	CH <sub>2</sub>	-Ph	4	18
<b>31</b>	CH <sub>2</sub>	-Ph( <i>o</i> -CO <sub>2</sub> Me)	0.002	0.5

**Table 1.6:** Improvements in potency from compound 26.

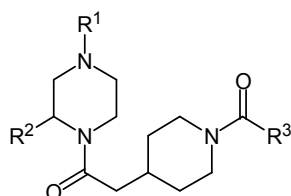
These compounds are some of the most active that have been reported in the literature so far, although no information is yet available regarding their *in vivo* activity or pharmacological properties.

High throughput screening has been also used by a number of other research groups and companies to identify lead compounds. Another significant example of this is compound **32**, which was identified by the Schering-Plough Corporation as an inhibitor of 17 $\beta$ -HSD3, which when its activity was tested upon the human 17 $\beta$ -HSD3 enzyme, it exhibited an IC<sub>50</sub> value of 2.5 nM (Figure 1.9).<sup>46</sup>



**Figure 1.9:** Lead compound identified by the Schering-Plough Corporation.

This led to the synthesis of a large number of compounds in this and related series, which subsequently resulted in a number of patents in 2003/2004.<sup>47-50</sup> The patents cover a wide range of modifications to the initial lead, some of which are shown in Table 1.7.

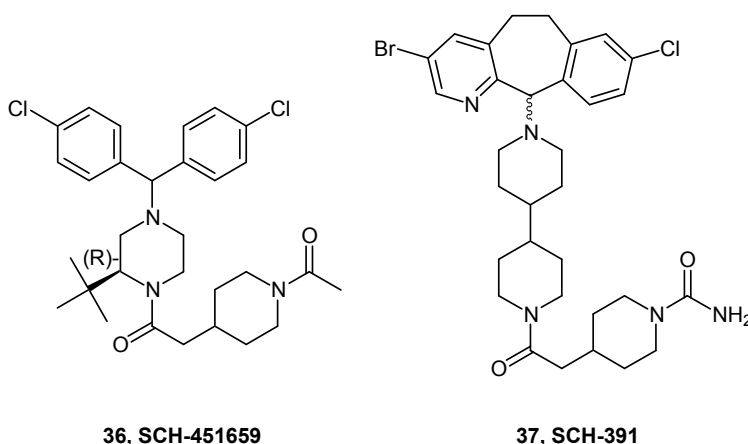


Compound no.	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	IC <sub>50</sub> (nM)
<b>32</b> <b>(Initial Lead)</b>		CH <sub>2</sub> CH <sub>2</sub> OCH <sub>3</sub>	NH <sub>2</sub>	2.5
<b>33</b>		CH <sub>2</sub> CH <sub>2</sub> OCH <sub>3</sub>	NH <sub>2</sub>	2600
<b>34</b>		CH <sub>2</sub> CH <sub>2</sub> OCH <sub>3</sub>	NH <sub>2</sub>	1.1
<b>35</b>		(CH <sub>3</sub> ) <sub>3</sub>	NH <sub>2</sub>	0.01
<b>36</b> <b>(SCH-451659)</b>		(CH <sub>3</sub> ) <sub>3</sub>	CH <sub>3</sub>	0.005

**Table 1.7:** Some of the modifications to the original lead compound.<sup>46</sup>

Modifications of the aryl substituent, R<sup>1</sup> led to the inclusion of a more flexible dichloro substituted diaryl group, which caused an improvement in potency (compounds **32** and **34**). These halogen substitutions were shown to be beneficial for activity, **34**.<sup>46</sup> Further investigations led to the introduction of an (*S*)-lipophilic *t*-butyl substitution at the 2 position of the piperazine ring, which also improved potency, **35**. This potency was further improved by the introduction of acetyl group on the piperidine moiety. This led to SCH-451659 (Figure 1.8, **36**), which has now become a potential clinical candidate, although its formal clinical development status, if any, has not been disclosed. This compound (**36**, SCH-451659) is also detailed in Chapter 2.

Efficacy studies, using compound **36**, in cynomolgus monkeys showed promising results, leading to a 50% reduction in serum testosterone levels, an 85% decrease in testes testosterone levels and a 20% decrease in prostate weight after 4 weeks dosing at 15 mg/kg, BID, p.o.<sup>51</sup> Similar studies on a related compound (SCH-391, **37**, Figure 1.10), showed that the inhibition of 17 $\beta$ -HSD3 led to the inhibition of androgen-dependent Shionogi tumour growth in nude mice (75 mg/kg, BID, i.p.).<sup>52</sup> These results illustrate the proof of concept in higher mammals, showing that inhibition of 17 $\beta$ -HSD3 is a valid target for prostate cancer.



**Figure 1.10:** Compounds tested for efficacy: SCH-451659 (**36**) and SCH-391 (**37**).

It can be seen that there is a great deal of interest in the design and synthesis of potent inhibitors of 17 $\beta$ -HSD Type 3 from both academic and industrial researchers. Much more work is required before a 17 $\beta$ -HSD Type 3 inhibitor will be seen in clinical trials or eventually as a potential treatment of choice in the clinic for prostate cancer.

## 1.12 Aims of This Thesis

The aims of this project are to design and synthesise novel 17 $\beta$ -HSD Type 3 inhibitors. It is anticipated that inhibitors of 17 $\beta$ -HSD Type 3 would block the biosynthesis of testosterone and therefore be an effective treatment for prostate cancer and other androgen dependent diseases. It is hoped that this project will produce compounds which are potent inhibitors and are selective over other isozymes in the 17 $\beta$ -HSD enzyme family, particularly 17 $\beta$ -HSD Types 1 and 2.

This project will utilise many modern medicinal chemistry techniques. The first step is that a lead series of compounds needs to be identified. In order to do this most effectively, a survey of competitor compounds will be undertaken and from this a pharmacophore can be established. It is hoped that this will provide an effective starting point for synthesis.

The project will also use modern computational chemistry techniques to assess the potential success of compounds and to establish areas of space that warrant further investigation. A crystal structure of 17 $\beta$ -HSD Type 3 is not currently available so a homology model will be constructed from the available crystal structure of a closely related enzyme. This will enable potentially significant interactions between the enzyme and inhibitors to be identified.

During this project the main focus will be the development of an effective SAR between potential inhibitors and the 17 $\beta$ -HSD Type 3 enzyme. The compounds synthesised will be assessed for their biological potency using a whole cell assay developed by colleagues at Imperial College. As biological results are obtained a QSAR should be constructed, thus aiding project progression further.

This project aims to design and synthesise compounds that would be suitable drug candidates. Therefore the inhibitors must have suitable molecular weights, lipophilicity and other properties. Only non-steroidal templates will be explored as this should help to limit any possible androgenic activity of the compounds.

Initially it is planned that the compounds synthesised will not contain a chiral centre. This will also negate the need for the use of asymmetric chemistry. As the project progresses chirality is likely to become an important issue that will need to be investigated using chiral separation or asymmetric chemistry as required.



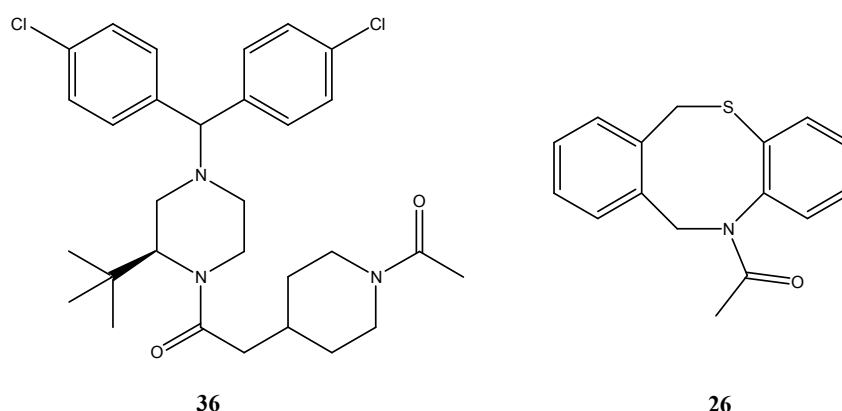
Overall, the aim of this project is to produce novel inhibitors of 17 $\beta$ -HSD Type 3 which exhibit an IC<sub>50</sub> below 100 nM and which could ultimately be applied to an unmet medical need.

## Chapter 2

### Selection and Synthesis of Compounds 26 and 36 as Positive Control Compounds

#### 2.1 Selection of Positive Control Compounds

During the development and validation of the biological assay used in this project it was necessary for a positive control compound to be available. A positive control compound is required to cause a known effect in the biological assay so, in this case, compounds that were known to exhibit inhibitory activity on 17 $\beta$ -HSD Type 3 were needed. It has been previously stated that there are many 17 $\beta$ -HSD3 inhibitors reported in the chemical literature,<sup>28, 46</sup> and a selection of suitable compounds were identified for further investigation, including compound **36**, a compound detailed by Schering Plough,<sup>47</sup> and compound **26**, which is a compound designed by Bristol-Myers-Squibb, (Figure 2.1).<sup>45</sup>



**Figure 2.1:** Positive control compounds, **36**<sup>47</sup> and **26**.<sup>53</sup>

#### 2.2 Identification and Synthesis of Compound 36

As previously mentioned, Schering-Plough have published a number of patents and publications over the past few years regarding novel non-steroidal 17 $\beta$ -HSD Type 3 inhibitors.<sup>46-52</sup> A large number of compounds has been synthesised during their medicinal chemistry programme; one of the most potent is SCH-451659, (**36**, Figure 1.8).<sup>46</sup> This compound is a potential clinical candidate and has showed promising proof of concept results in animal models, for more details see Chapter 1.<sup>51</sup> As one of the most potent inhibitors detailed in the chemical literature, compound **36** was the logical choice for an initial biological control compound.

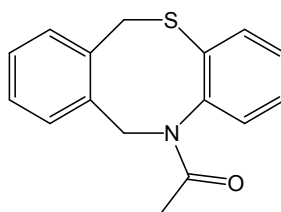
The experimental procedure for the synthesis of this compound was detailed in a patent from Schering-Plough.<sup>47</sup> This synthetic route was repeated by Dr G. M. Allen and compound **36** was subsequently used as the positive control in the biological assay development and screening.

However, during the course of the project it was found that compound **36** had an inhibitory activity that was several orders of magnitude more potent than the novel compounds synthesised as part of this project. The highly potent nature of compound **36** meant that a consistently low IC<sub>50</sub> was obtained (approx 2-6 nM), and this was not in a similar range to the in-house compounds, meaning any possible assay inconsistencies may have been masked by this highly potent activity, so an alternative positive control compound was required.

### 2.3 Identification and Synthesis of Compound 26

It was desired that a positive control could be identified, which exhibited activities in the same range as the best of the novel, in-house compounds, which had approximate IC<sub>50</sub> values of between 300 and 900 nM (see Chapters 6-10). This means that any changes exhibited by the standard between assay runs, would show up inconsistencies within the biological assay.

A search of the chemical literature exposed a recent paper by Fink *et al*<sup>45</sup> detailing a novel series of 17 $\beta$ -HSD type 3 inhibitors based around the tetrahydrodibenzazocine core. They identified compound **26** (Figure 2.2 and Table 1.5) as a compound in the series with moderate activity. This was discussed in more detail in Chapter 1.



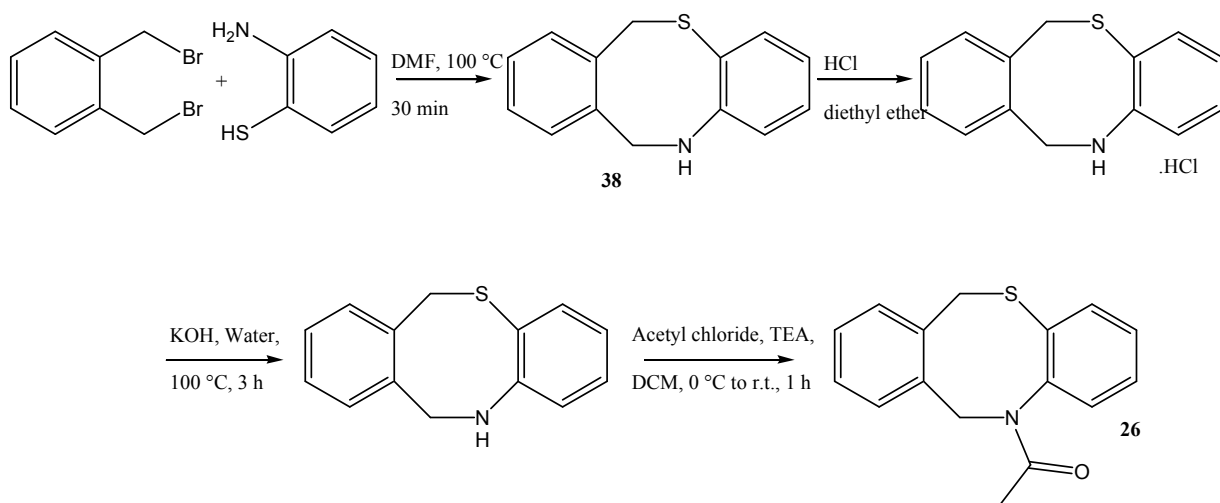
**Figure 2.2:** Compound **26**

Based on the activities reported by Fink *et al*,<sup>45</sup> it was decided that compound **26** would be a good choice for a new positive control for use in the biological assay. This was because

the reported activity (cellular IC<sub>50</sub> = 0.32 μM) is in the region displayed by our novel in-house compounds and also in view of the facile two step synthesis that was possible from commercially available starting materials.<sup>54, 55</sup>

The synthesis of compound **26**, was first reported by Yale *et al* in 1972.<sup>54</sup> This paper looked at the synthesis of novel polycyclic heterocycles, but did not examine any potential biological activity the compounds may possess.

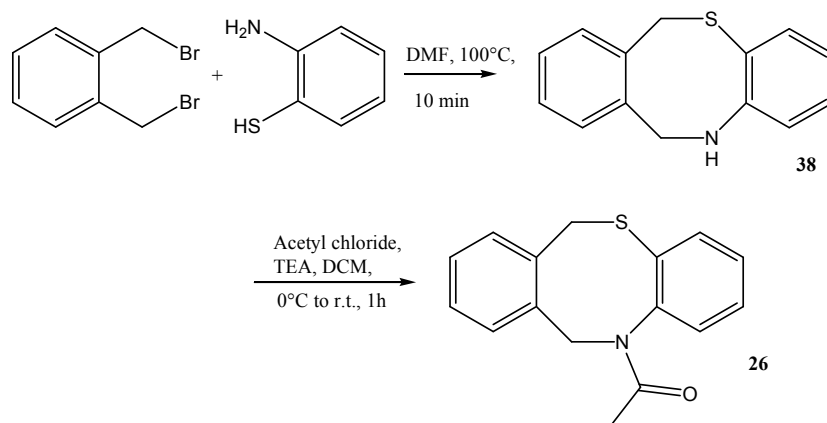
Following the original synthetic strategy (Scheme 2.2)<sup>54</sup> a mixture of 2-aminothiophenol and α,α'-dibromo-o-xylene was added to a solution of DMF at 100 °C. This was heated for 30 minutes, before being cooled and evaporated to dryness. Following an extraction between NaHCO<sub>3</sub> and diethyl ether a brown oil was isolated (**38**). By treatment with HCl in diethyl ether, this was converted into the HCl salt, which was isolated by filtration. The salt was dissolved in water and KOH was added. This was heated to 100 °C for 3 h, cooled and extracted with EtOAc to yield the free amine. The free amine was then reacted with acetyl chloride to yield the desired product, (Scheme 2.1). However, this initial synthetic strategy only led to 11 % yield overall. When compared with the original paper, which reported an overall yield of approximately 60 %, this shows that a significant improvement in the synthesis was required.



**Scheme 2.1:** Initial synthesis of compound **26**. Overall Yield: 11 %

It was felt that the formation of the HCl salt and the following release of the amine were unnecessary steps, which could be replaced by simple column chromatography after the first step. This increased the overall yield to 32 %. However, a vast improvement in yield

was obtained when this purification step was removed entirely and the acetylation was carried out on the crude product from the first step, **38**. This, and reducing the reaction time in the first step to just 10 minutes, led to an increase in yield to 73 % overall, (Scheme 2.2). This meant that this positive control could be easily and rapidly synthesised in relatively large quantities to be used continuously in the biological assay.



**Scheme 2.2:** Improved synthetic route to compound **26**. Best overall yield: 73 %

## Chapter 3

### Biological Evaluation

#### 3.1 Introduction

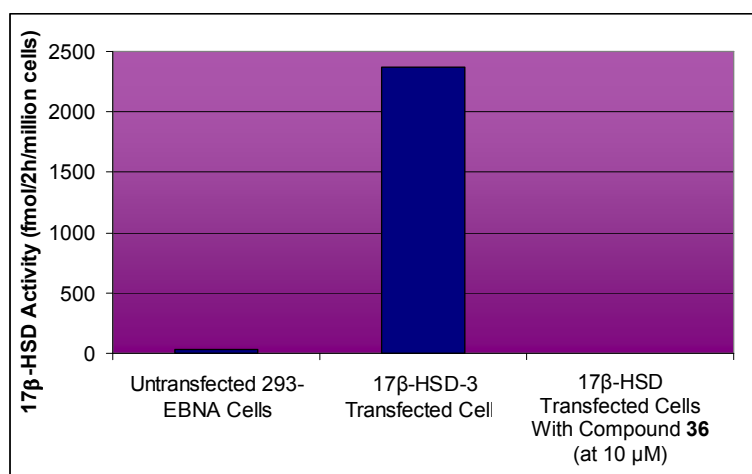
The compounds synthesised needed to be assessed for their inhibitory activity upon 17 $\beta$ -HSD Type 3, in order for a successful Structure Activity Relationship (SAR) to be developed and thus allow the project to progress and more active compounds to be designed and synthesised. Initially, all the compounds were assessed for 17 $\beta$ -HSD3 inhibitory activity by analysis in a whole cell assay. It measured and compared the amount of testosterone produced by the cells in the presence and absence of potential inhibitors, and therefore allowed a comparison of enzymatic activity with those cells that were not exposed to any potential inhibitors.

There are many ways of assessing testosterone formation, including direct and indirect methods. During the course of this project two different assay formats have been utilised: direct measurement of testosterone using a Thin Layer Chromatography (TLC) based assay and an indirect DELFIA<sup>®</sup> assay (Dissociation Enhanced Lanthanide Fluorescence Immunoassay).

#### 3.2 Thin-Layer Chromatography (TLC) Whole Cell Assay

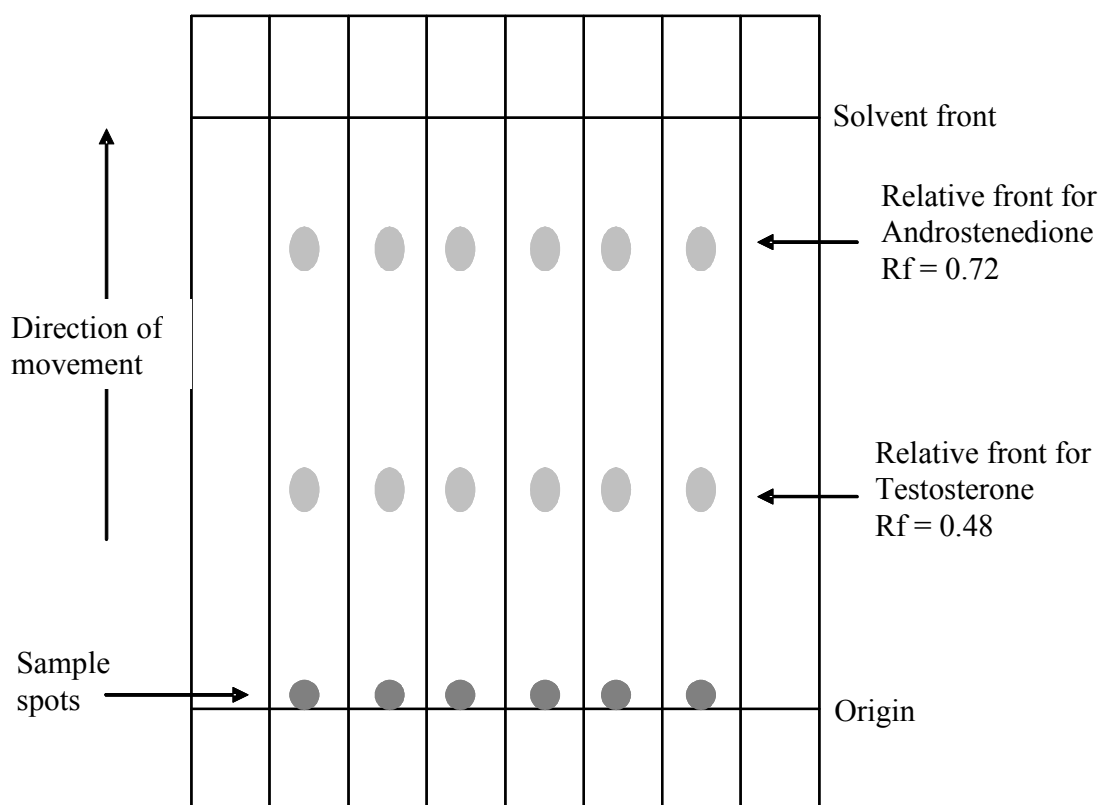
This assay was developed and carried out by colleagues at St Mary's Hospital Medical School (Imperial College, London).

This assay is a whole cell assay, using a transfected 293-EBNA cell line (derived from embryonic kidney cells) with stable expression of human 17 $\beta$ -HSD Type 3 (293-EBNA[HSD3]). Non-transfected cells had negligible 17 $\beta$ -HSD3 activity, but when transfected with 17 $\beta$ -HSD Type 3 cDNA in pCEP4, the transfected cells exhibit a huge over-expression of 17 $\beta$ -HSD3 (Figure 2.1). The over-expression of 17 $\beta$ -HSD3 results in a correspondingly large increase in 17 $\beta$ -HSD3 activity, the production of testosterone from androstenedione (Figure 2.1). This activity was dramatically reduced when the cells were incubated with 10  $\mu$ M of compound **36**, showing the positive control compound to exert the effects expected (Figure 2.1).



**Figure 2.1:** A bar chart to show the increase in 17β-HSD3 activity in the transfected cells and the inhibition of activity caused by compound **36**.

The 293-EBNA[HSD3] cells were plated at ~50,000 cells/well in 24 well plates, in complete growth medium (including 10% FBS, but without G418 or Hygromycin B). After 48 hours 2-3 nM  $^3\text{H}$ -androstenedione was added, in the presence or absence of the potential inhibitor (initially at 10 μM concentration), and the cells were incubated at 37 °C. The samples were subjected to extraction with diethylether, spotted onto silica TLC plates and androstenedione and testosterone were separated using a 4:1 DCM: EtOAc mobile phase (Figure 2.2). Good separation is achieved using this solvent system, with an  $R_f$  of 0.72 for androstenedione compared to 0.48 for testosterone. After drying, the testosterone spots were identified, isolated, removed and placed into a scintillation vial. Methanol and scintillation fluid were added and the samples were subjected to scintillation counting.



**Figure 2.2:** Layout of the TLC plate used for the assay, (using 4:1 DCM: EtOAc solvent system).

The inhibitory activities (measured as the percentage inhibition at 10  $\mu\text{M}$ ) of the test compounds were assessed by comparison with the control (where no test compound was added). The same assay was used to measure the  $\text{IC}_{50}$  of selected compounds. Compounds were applied to the cells at a range of concentrations (commonly 6 different concentrations between 0.001  $\mu\text{M}$  and 10  $\mu\text{M}$ ) and data analysis allowed the  $\text{IC}_{50}$  for each test compound to be calculated.

The TLC assay was validated using compound **36**, the initial positive control compound. The results obtained using compound **36** were excellent. It exhibited a low  $\text{IC}_{50}$  of between 2 nM and 7 nM. Schering-Plough reported compound **36** to exhibit an  $\text{IC}_{50}$  of 0.005 nM, which is considerably lower than the results obtained in this assay. However, the assay used by Schering-Plough is an enzymatic assay and therefore it would not be expected to produce the same results as the whole cell TLC assay. The whole cell assay results will be influenced by the ease of which the test compound can pass through the cellular membrane and the compound can also be subject to metabolism or excretion from the cell. All of these factors can cause a lower apparent activity. Most importantly, in the TLC assay the



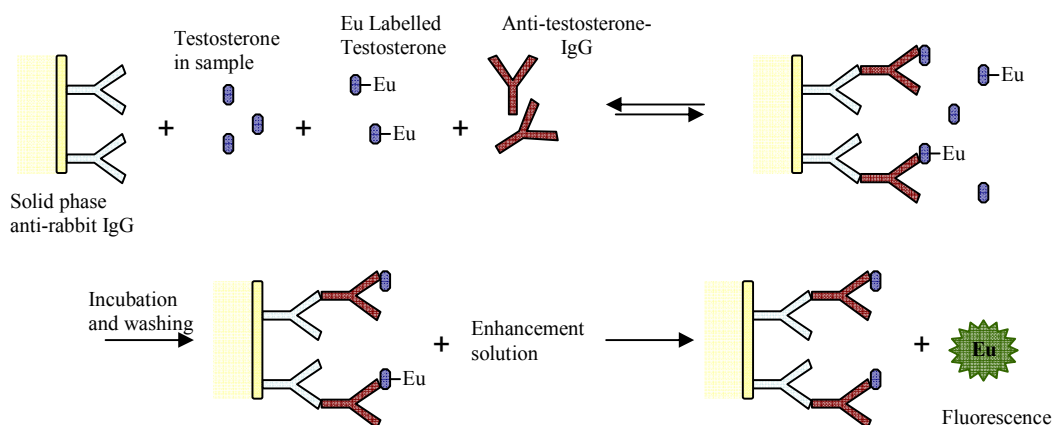
results obtained for compound **36** were consistent, and thus showed the assay to be reliable and reproducible.

The TLC assay was the original assay to be developed for the project, and it produced consistent, reliable results. However, the main disadvantage with this assay is the time and manpower required. This assay is laborious and therefore cannot be transferred to a high-throughput assay format. Some aspects of this assay can be automated, for example automated TLC spotters can be used, however, even with this automation the TLC assay is low throughput. For these reasons investigations were carried out into the development of a new high-throughput assay format. These developments led to the introduction of the DELFIA<sup>®</sup> assay.

### **3.3 DELFIA<sup>®</sup> Assay**

The DELFIA<sup>®</sup> assay used for this project is the Perkin Elmer DELFIA<sup>®</sup> Testosterone Assay, which was originally designed for the quantitative determination of low level testosterone in human serum samples.<sup>56</sup>

The DELFIA<sup>®</sup> Testosterone assay is a fluoroimmunoassay, based upon the competition between europium labelled testosterone and free testosterone within the sample for polyclonal anti-testosterone antibodies (Figure 2.3). The testosterone contained within standard solutions, control solutions and serum samples inhibit the binding of labelled testosterone to the antibodies to varying extents, dependent upon concentration. A second antibody, which binds to the anti-testosterone antibody, is attached to the solid phase of the 96 well plate, thus meaning separation of bound and unbound antigen is experimentally straightforward. Enhancement solution is finally added to the wells, which dissociates europium ions from the labelled testosterone, these then form highly fluorescent chelates with components of the enhancement solution. This fluorescence is measured and is inversely proportional to the concentration of testosterone in the original sample or standard.<sup>57-59</sup> A calibration curve is constructed from the standards supplied with the test kit; this is used to obtain testosterone concentrations, from which the percentage inhibition can be obtained.



**Figure 2.3:** Principle of DELFIA<sup>®</sup> Assay.<sup>8</sup>

The main advantage of this kit is that it is a high-throughput assay. The 96-well plate format means that at one concentration up to 40 different compounds can be analysed to obtain a percentage inhibition result in one assay, thus meaning that results are obtained rapidly and project progression should be swift.

### 3.3.1 Development and Validation of DELFIA<sup>®</sup> Assay

The DELFIA<sup>®</sup> assay was developed by Argenta Discovery Ltd,<sup>60</sup> using the 293-EBNA[HSD3] cell line derived at St Mary's, London. Further development and results were obtained by colleagues at IPSEN Ltd, Paris.

Argenta Discovery were supplied with three compounds to use for assay validation; these compounds were compound **36**, the Schering-Plough compound,<sup>47</sup> and compounds **100** and **101** (see Chapter 7), which were, at the time, the best novel, in-house compounds we had synthesised (see Chapter 7).<sup>18</sup> These three compounds were analysed by Argenta Discovery Ltd in both the newly developed DELFIA<sup>®</sup> Assay and in the original TLC assay, (Table 2.1). The IC<sub>50</sub> values obtained for compound **36** are comparable in both assay formats; however the results for compounds **100** and **101** were less promising, as both showed a clear difference in IC<sub>50</sub> values in the two assay formats. It appeared that the two compounds are five to tenfold less potent in the DELFIA<sup>®</sup> assay, when compared with the TLC assay. It was not possible to obtain the sensitivity shown in the TLC assay using the DELFIA<sup>®</sup> assay format.

Compound	TLC (IC <sub>50</sub> )	DELFI <sup>®</sup> A (IC <sub>50</sub> )
<b>36</b>	1 nM	6 nM
<b>100</b>	7 μM	~ 80 μM
<b>101</b>	3 μM	~ 37 μM

**Table 2.1:** IC<sub>50</sub> values for positive control compounds during assay validation by Argenta.  
(Note: 50 nM substrate used).

Many possible explanations for the differences in sensitivity between the two assay formats have been hypothesised and explored, including substrate concentration, cell density, the presence of antibiotics, solvent choice and DMSO concentration. None of the investigations carried out has been able to identify the cause of the disparity.

The assay underwent transfer from Argenta Discovery to IPSEN, where further optimisation and validation took place. Many of the test compounds were then subjected to routine primary screening to establish the percentage inhibition exhibited by the compounds. However, problems were still encountered regarding the sensitivity of the DELFI<sup>®</sup>A assay, as the vast majority of the compounds tested were found to be inactive. Some of the compounds that were shown to be inactive in this assay had previously been shown to be active in the TLC assay (Table 2.2 and individual Chapters 6-8). For example, compound **165** (see Chapter 8) showed a highly promising percentage inhibition of 91% (at 10μM) in the TLC assay, with a moderate IC<sub>50</sub> of 3.9 μM, but when the compound was tested in the DELFI<sup>®</sup>A assay, no activity was observed and a percentage inhibition result of -51% (at 10μM) was obtained.

Compound no.	Percentage Inhibition (%, at 10 $\mu$ M)		IC <sub>50</sub> ( $\mu$ M)	
	DELFI <sup>®</sup>	TLC	DELFI <sup>®</sup>	TLC
<b>36</b>	99	99	0.015	0.0024
<b>63</b>	-52	51	a	a
<b>101</b>	-23	89	37	0.7
<b>123</b>	94	94	3	0.383
<b>126</b>	-10	87	a	0.9
<b>131</b>	-33	86	a	a
<b>162</b>	-10	55	a	a
<b>165</b>	-51	91	a	3.9

**Table 2.2:** Selected biological data, to illustrate discrepancies between the sensitivity of the DELFI<sup>®</sup> assay and the TLC assay. (<sup>a</sup> Not determined)

To assess the reliability of a biological assay, a positive control compound is used, as any change in the results would highlight discrepancies in that assay. Up to this point compound **36** had been used as the biological control. However, as mentioned previously (Chapter 2), it is a highly potent compound and because of this, small alterations in assay format may not affect the overall result. It is desirable to have a positive control compound which has similar activity to the compounds that are being analysed. This led to the introduction of compound **26** as the new biological control (see Chapter 2). When compound **26** was tested, the clear difference in activity observed with the TLC and the DELFI<sup>®</sup> assay remained. In the TLC assay compound **26** exhibited an IC<sub>50</sub> of 590 nM, compared to an IC<sub>50</sub> of 1000 nM in the DELFI<sup>®</sup> assay. The causes of these differences remain unknown.

It seemed that although the DELFI<sup>®</sup> assay was able to produce reliable results for the highly potent Schering-Plough positive control (**36**), the assay was unable to produce satisfactory results for the in-house inhibitors, as the potency of these is lower as the lead series is developed. Once the project has progressed to a stage where the potency of the novel compounds approaches the activity of compound **36**, the DELFI<sup>®</sup> assay may become suitable for this project. However, it was decided that at the current stage of the project, the DELFI<sup>®</sup> assay was not suitable, so attention was diverted back to the TLC assay, and the results obtained from this assay. All results quoted in further Chapters were obtained from the TLC assay run at St Mary's Hospital.

Unfortunately, due to time restrictions no further progress has been made in the development of a suitable assay. This means that the biological evaluation of many completed targets has not yet been carried out, delaying effective project progression. These compounds will be evaluated after submission of this thesis.

Where biological results have been received these will be discussed in the individual Chapters 6-10.

## Chapter 4

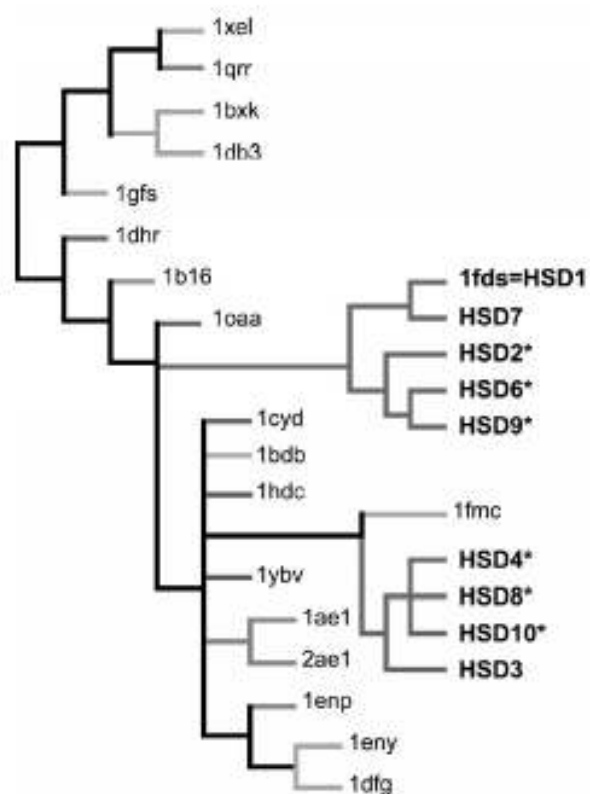
### Computational Modelling

#### 4.1 Introduction

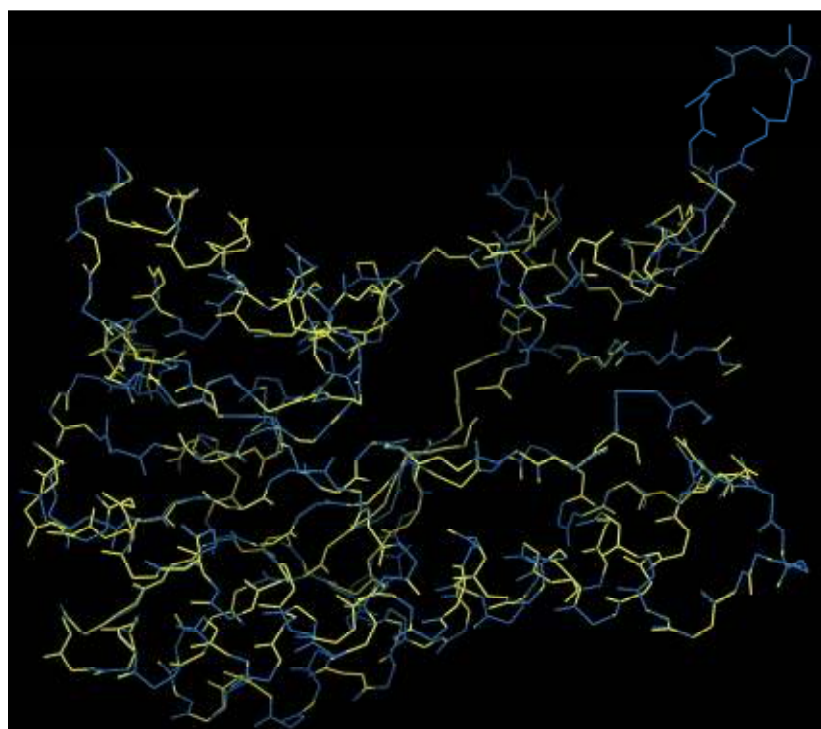
A detailed knowledge of a target binding site would significantly aid the design of potential inhibitors. In cases where enzymes have been crystallised it is possible to determine the structure of the protein and the nature of the binding of ligands by X-ray crystallography. However, in the case of 17 $\beta$ -HSD Type 3 an X-ray crystal structure is not available, as the enzyme is membrane bound and crystallisation is therefore difficult. The amino acid sequence of 17 $\beta$ -HSD Type 3 is known (Swiss-Prot identifier P37058, entry name DHB3-Human) and this allows a homology model to be constructed.<sup>34</sup> This procedure develops a three-dimensional model from a protein sequence based on the structures of homologous proteins. Please note this work was carried out by Dr A Smith and Dr M Trusselle.

#### 4.2 Construction of the 17 $\beta$ -HSD Type 3 Homology Model

The structure of an analogous protein was required for use as the template for a homology model of 17 $\beta$ -HSD Type 3. In order to choose the correct template it is necessary to look at the evolutionary path of the protein of interest, enabling the most closely related protein structure to be used. A phylogenetic tree reported by Breitling *et al* was used and this is shown in Figure 4.1.<sup>61</sup> Those enzymes with an available X-ray structure are represented by their PDB code.<sup>62, 63</sup> From the phylogenetic tree it can be seen that the 17 $\beta$ -HSD family is split into two distinct branches. The first branch contains types 1, 2, 6, 7 and 9, the second branch contains types 3, 4, 8 and 10 and so this is the branch of interest. There are no crystal structures currently available for types 4, 8 or 10 and so the closest available crystal structure is that of 1FMC (7 $\alpha$ -hydroxysteroid dehydrogenase from *Escherichia coli*).<sup>64</sup> The use of 1FMC as a template allowed a homology model to be constructed (Figure 4.2).



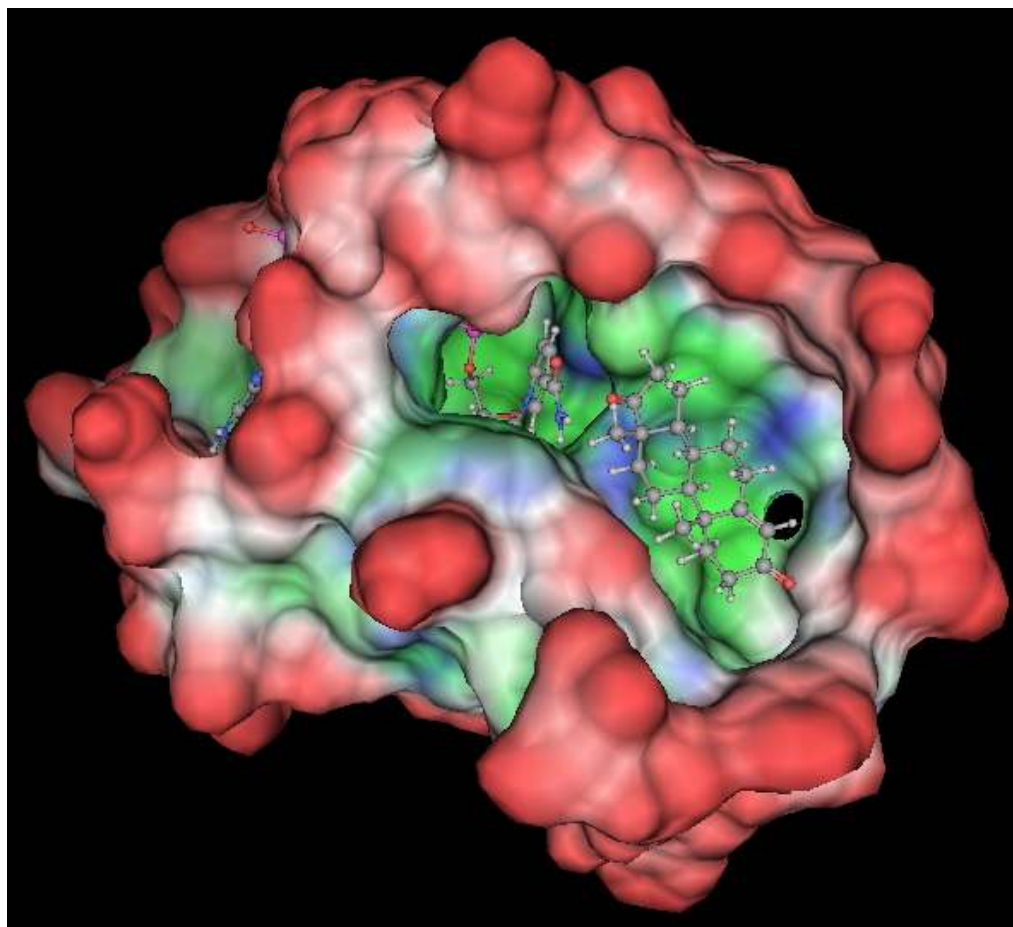
**Figure 4.1:** Phylogenetic Tree for the 17β-HSD family.



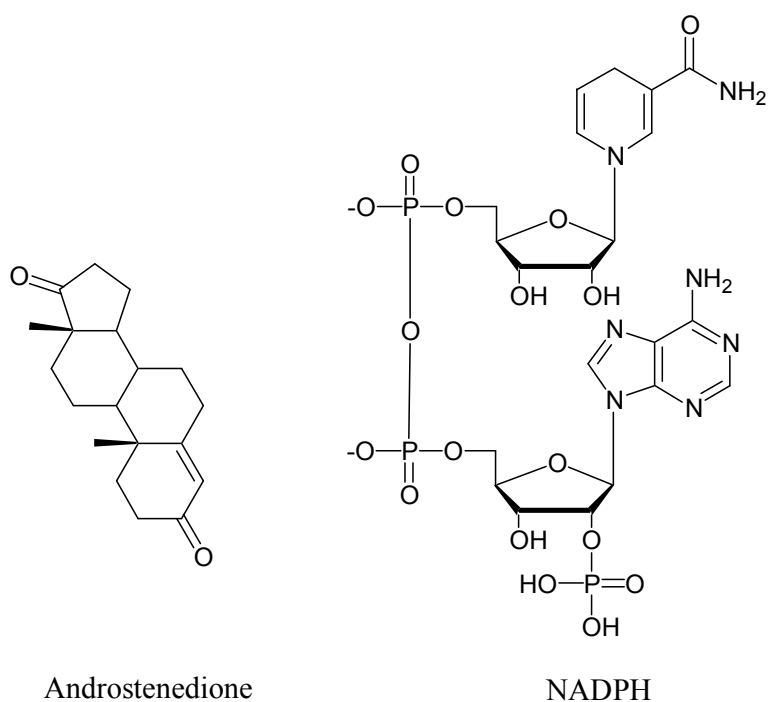
**Figure 4.2:** Constructed Homology Model. (Template shown in yellow, Homology model shown in blue).

### 4.3 Use of the 17 $\beta$ -HSD Type 3 Homology Model.

Androstenedione, the natural enzyme substrate, and NADP(H), the co-factor, are shown docked into the homology model (Figure 4.3). It can be seen that the C<sub>17</sub> carbonyl group on androstenedione is orientated towards the nicotinamide ring of the co-factor in the catalytic region.

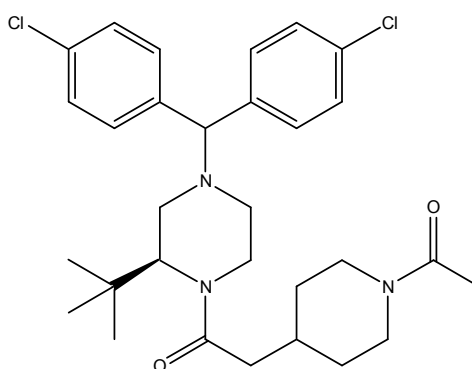
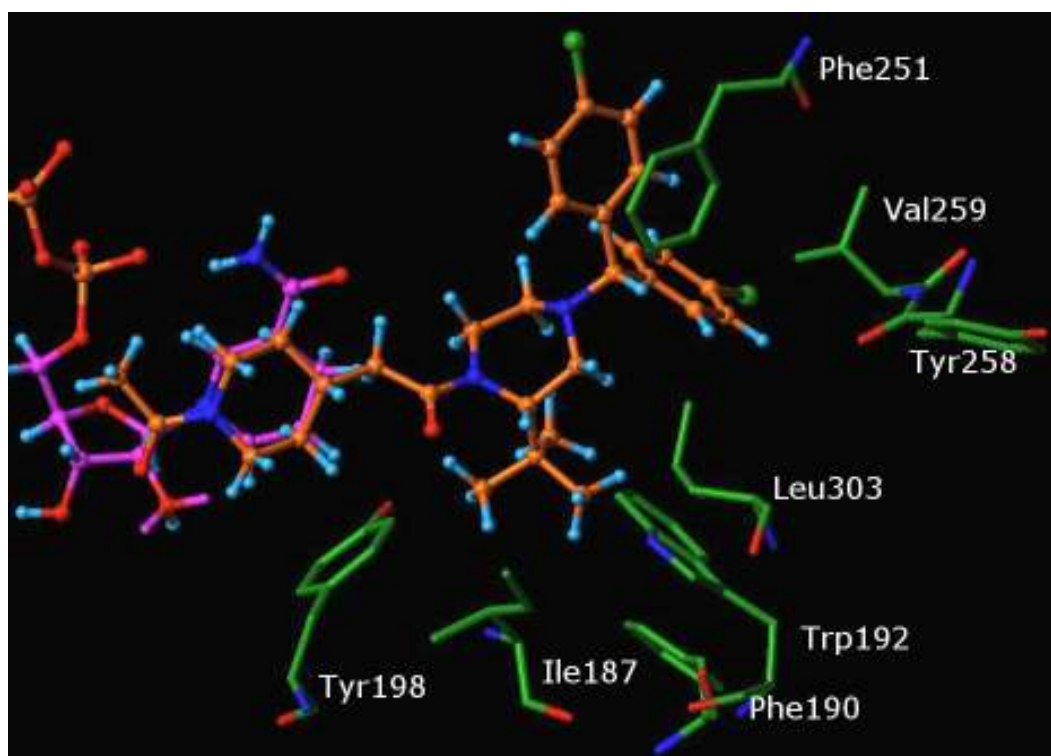






**Figure 4.3:** The 17 $\beta$ -HSD Type 3 homology model with androstenedione and NADPH docked. The enzyme surface colours indicate lipophilic (green) to hydrophilic (red).

It can be seen from Figure 4.4 that compound **36**, a potent 17 $\beta$ -HSD Type 3 inhibitor from Schering-Plough,<sup>47</sup> appears to bind tightly with the active site of the homology model. An association can be observed between the co-factor and the acetamide at the base of compound **36**. The *t*-butyl group occupies a lipophilic pocket formed by Ile-187, Phe-190, Tyr-198 and Leu-303. It can also be seen that the diphenylmethylene headgroup interacts effectively with a hydrophobic pocket of the enzyme.



**Figure 4.4:** Key interactions between compound **36** and amino acid residues in the active site of 17 $\beta$ -HSD Type 3, identified from the docking calculations

Once the homology model had been constructed and optimised it was then suitable for use in the design of novel 17 $\beta$ -HSD Type 3 inhibitors. Structures of interest were examined using the homology model and the docking of potential inhibitors enabled important interactions to be estimated and regions of space to be identified.

It was hoped that this information could be used to predict or explain differences in biological activity. It should be noted though, that a homology model can only be used as a guide. Although every care was taken to ensure the accuracy of its design, it cannot be

assumed to be conclusive. It is possible that the shape of the active site may not be accurately represented by a homology model and the fluctuations that occur in enzyme active sites are not fully accounted for by the docking program. An X-ray crystal structure would be more reliable; however, as this is not currently available the homology model was used as a tool in structure based drug design.

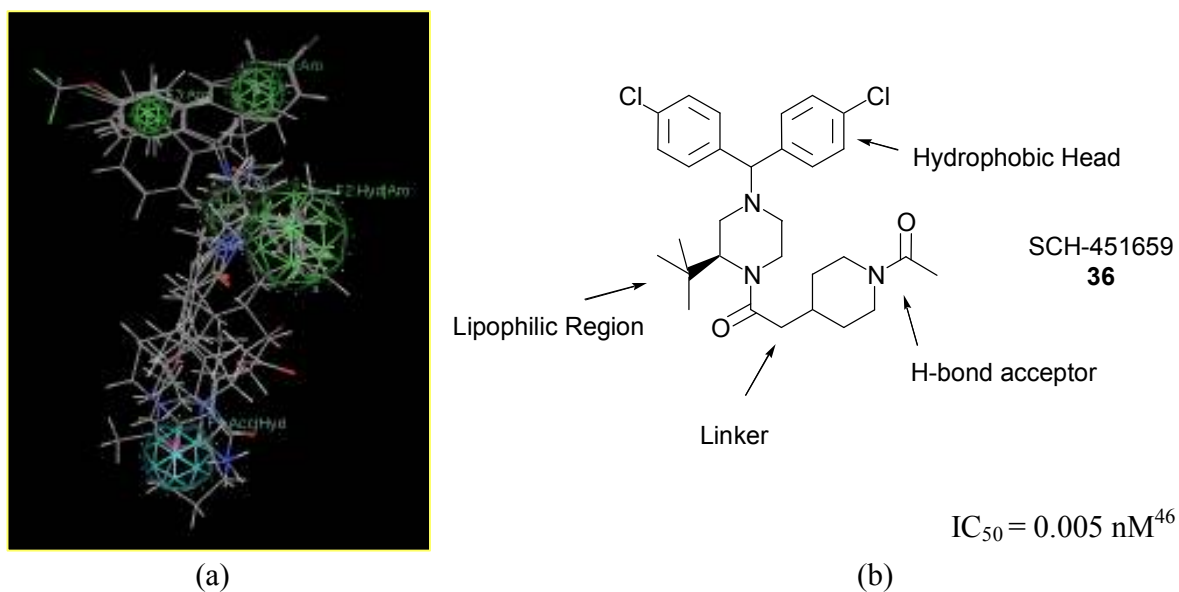
## Chapter 5:

### Development of Initial Lead Series

It has been hard to gather detailed information concerning the Schering-Plough non-steroidal 17 $\beta$ -HSD Type 3 inhibitors, as the patents contain no specific biological data.<sup>47-50</sup> From the patents it was possible to identify a selection of compounds which were thought to be the most promising. From this selection, SCH-451659 (**36**) was selected as a positive control (see Chapter 2). This compound was synthesised and subjected to biological evaluation (see Chapter 3), which showed SCH-451659 (**36**) to be a highly potent 17 $\beta$ -HSD Type 3 inhibitor. The activity of this series of compounds was therefore confirmed. More information was subsequently published by Schering-Plough regarding SCH-451659, (**36**) and other similar compounds,<sup>46, 51, 52</sup> thus providing further evidence that the series of compounds identified from the patent show potent 17 $\beta$ -HSD Type 3 inhibitory activity. Much information about other novel, potential inhibitors could be gained from these structures.

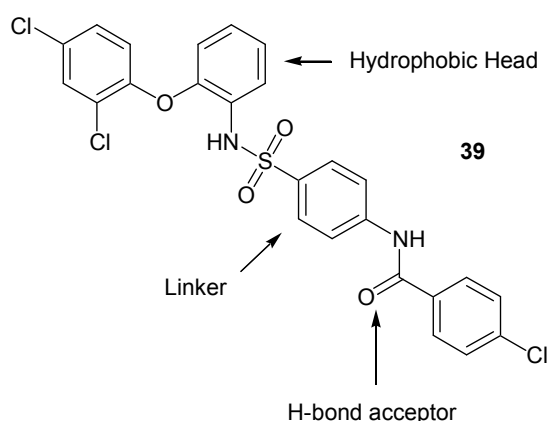
#### 5.1 Inhibitor Design

The structures from the Schering-Plough patents were examined and then were analysed to give a representation of a general pharmacophore required for 17 $\beta$ -HSD Type 3 activity (Figure 5.1a). This pharmacophore showed four distinct regions which were subject to little variation between the many compounds (Figure 5.1b). The four main regions were: (i) a hydrophobic head group, with varying structure and substitution, (ii) the lipophilic alkyl group on position two of the piperazine, (iii) a linking group and (iv) a hydrogen bond acceptor, most commonly a carbonyl group at the other end of the molecule to the hydrophobic head (Figure 5.1b).



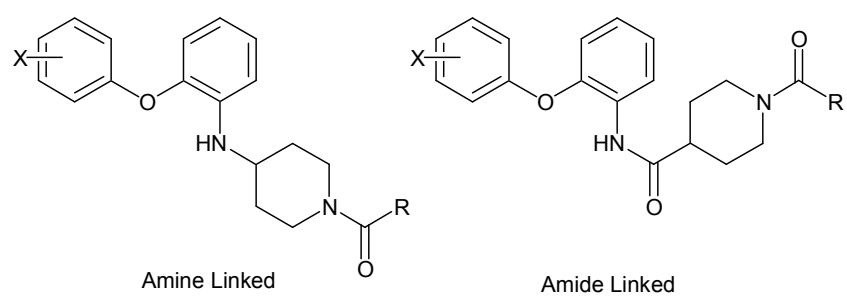
**Figure 5.1:** (a) The pharmacophore established from Schering-Plough compounds,<sup>47-50</sup>  
(b) SCH-451659 (**36**) showing the common regions in Schering-Plough compounds.

This pharmacophore information was used to identify suitable compounds from available commercial databases. One compound, **39** (Figure 5.2) was identified from the Maybridge database as fitting the desired pharmacophore hypothesis. The compound had the main desired regions from the pharmacophore; the hydrophobic head group was, in this case, a substituted diphenylether moiety.



**Figure 5.2:** The diphenylether compound identified from the Maybridge database (RFJ01724, **39**).





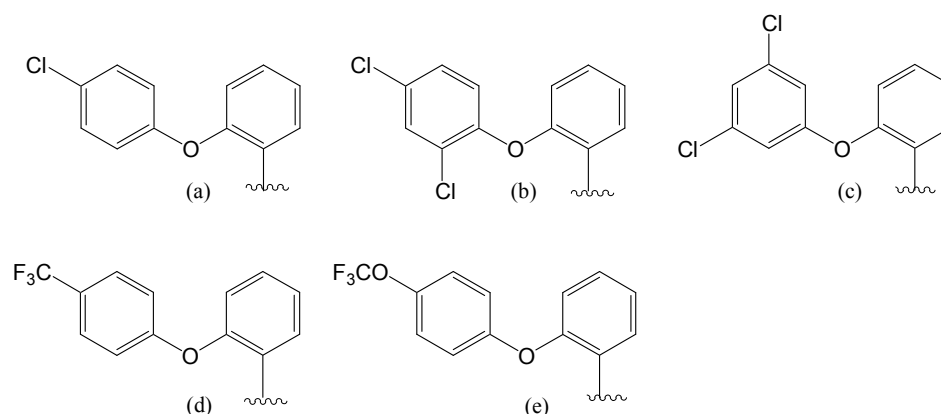
**Figure 5.4:** Amine and amide linked initial targets.

## Chapter 6:

### Synthesis and Biological Evaluation of the Amide Linked Series of Inhibitors

#### 6.1 Synthesis of Initial Targets

The synthesis of a novel series of amide linked target compounds was initiated (Scheme 6.1). It was hoped that this series of compounds would help to provide valuable knowledge about the size and shape of the active site and ultimately the tolerability of the enzyme to inhibitors. The use of an amide linking group determines not only the size, but also the geometry, polarity and electronics of the compounds. This series was also used to examine the effects of differing hydrophobic head group substituents and R groups in the hydrogen bond accepting region of the template (Figure 6.1). Using information regarding biologically active inhibitors from the Schering-Plough patents,<sup>47-50</sup> five different hydrophobic head substitution patterns were selected. These were 4-chloro, 2,4-dichloro, 3,5-dichloro, 4-trifluoromethyl and 4-trifluoromethoxy (Figure 6.1).

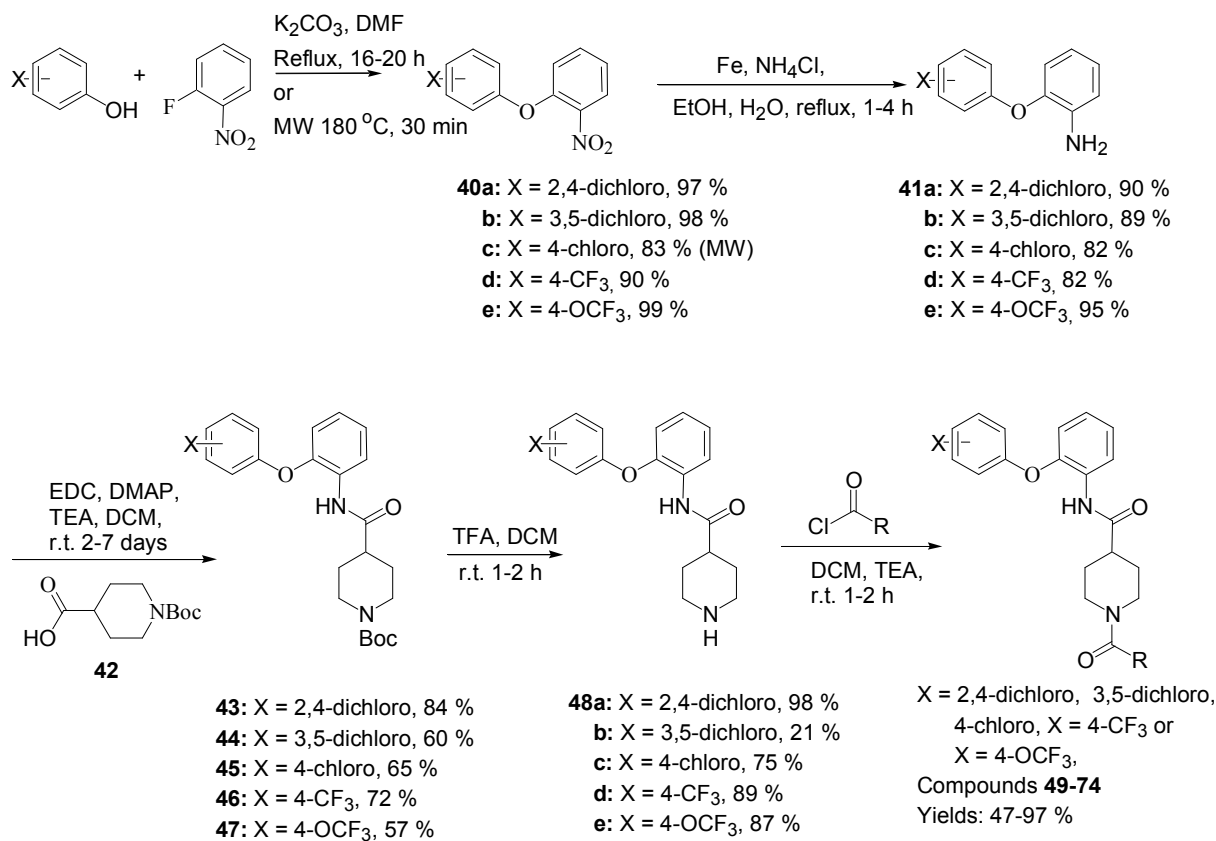


**Figure 6.1:** Selected diphenylether substitution patterns. (a) 4-chloro, (b) 2,4-dichloro, (c) 3,5-dichloro, (d) 4-trifluoromethyl, (e) 4-trifluoromethoxy.

To explore the SAR of the R group on the carbonyl mimic section of the compounds, six different alkyl groups of different sizes and electronics were selected to probe the steric constraints in this region. These were methyl, phenyl, cyclohexane, cyclopentane, *iso*-butyl and *iso*-valeryl. The *t*-Boc protected intermediates were also submitted for biological testing, to investigate the effects of the *t*-Boc moiety.



The synthesis of the amide linked targets is outlined in Scheme 6.1 and exhibited moderate to good yields.



**Scheme 6.1:** Synthesis of amide linked target compounds (**40-74**).

The first step in the synthetic route was the diphenylether formation (**40a-e**), which involved the coupling of the appropriate substituted phenol (e.g. 2,4-dichlorophenol) and 2-fluoro-1-nitrobenzene, in the presence of potassium carbonate. The mixtures were heated at reflux in DMF for between 16 and 20 hours.<sup>65</sup> This proved to be a very successful method with excellent yields obtained for many varying substitutions. However, the reaction time is very long, so a new method utilising microwave (MW) assisted synthesis was developed. The reaction between 4-chlorophenol and 2-fluoro-nitrobenzene, with potassium carbonate in DMF was heated using a CEM Discover® instrument,<sup>66</sup> for various times and at various temperatures. The results are shown in Table 6.1.

Time	Temperature (°C)	Conversion (%) <sup>a, b</sup>
10	160	75
20	180	77
30	180	91

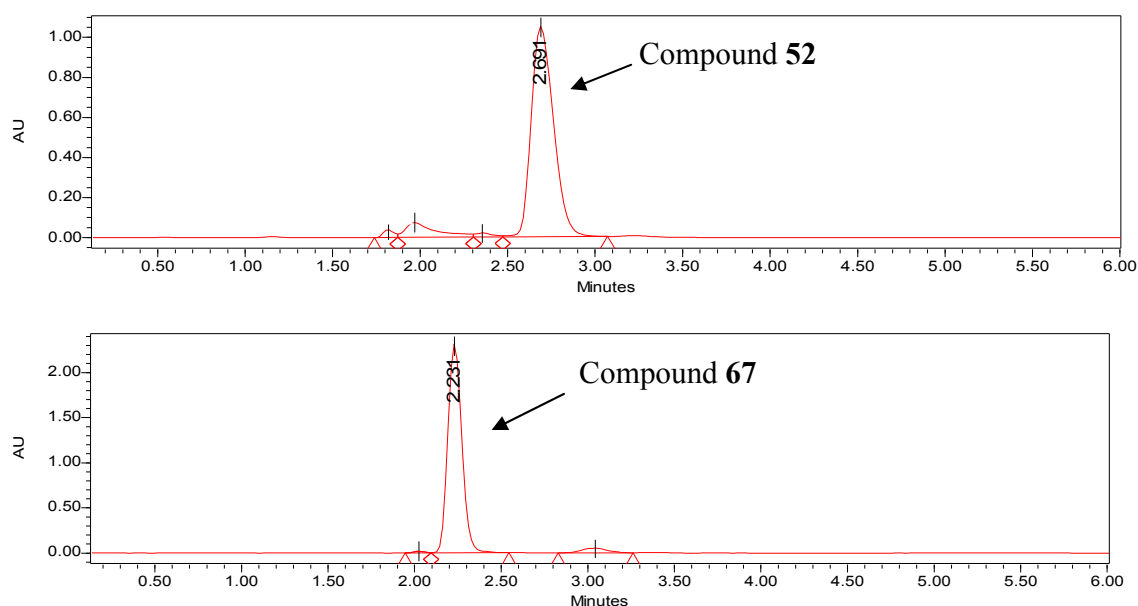
<sup>a</sup> Measured by HPLC

<sup>b</sup> Reaction conditions: 4-chlorophenol (2.25 mmol), 2-fluoronitrobenzene (1.5 mmol), K<sub>2</sub>CO<sub>3</sub> (2.7 mmol) in DMF (3 mL), were heated in a CEM Discover<sup>®</sup> instrument.

**Table 6.1:** Conversion results for varying times and temperatures.

It was found that the reaction had not reached completion when heated at 160 °C or 180 °C for 10 or 20 min. Completion was approached when the reaction time was increased to 30 min. The correct product was isolated in an 83 % yield using this MW technique. This is comparable to the original method, but with a reaction time greatly reduced from 18 h to 30 min.

The nitro group on the synthesised intermediates was reduced using iron powder and ammonium chloride in a mixture of ethanol and water at reflux to form the diphenylether aniline template (**41a-e**).<sup>65</sup> An amide coupling was now required and, although slow (reaction time 2-7 days), this step has allowed for the successful synthesis of 30 compounds in this series. Following the amide coupling, the compounds required the removal of the *t*-Boc protecting group by TFA, followed by the final synthetic step in this route; the amide formation with the appropriate acid chloride. Figure 6.2 shows an illustrative example of a HPLC trace for 2 compounds synthesised in this series, **52** and **67**.

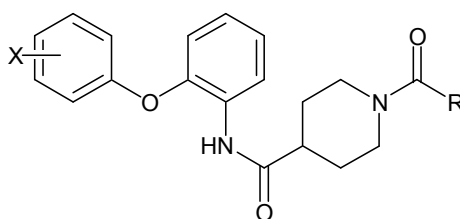


**Figure 6.2:** Illustrative examples of HPLC traces for compounds **52** and **67**, (solvent system: 90 % Acetonitrile in water at 1.0 mL/min).

The final step in this synthetic route could either be carried out using standard laboratory or parallel synthesis techniques. The initial methyl and phenyl derivatives were prepared using standard laboratory techniques, and the cyclohexane, cyclopentane, *iso*-butyl and *iso*-valeryl compounds were all prepared using parallel synthesis techniques. There was no significant change in yields when parallel synthesis techniques were used. However, they allow many more compounds to be made in a shorter time. This is essential in medicinal chemistry research, where many similar compounds need to be synthesised in order to successfully and rapidly establish an SAR between the compounds and the enzyme in question.

## 6.2 Biological Results and Discussion: Amide Linked Series.

Thirty amide linked target compounds were synthesised, Table 6.2 shows all the compounds synthesised. All the amine linked target compounds have been submitted for biological testing. Table 6.2 also shows the available biological data obtained from the TLC assay.



Compound Number	X	R	% Inhibition (at 10 $\mu$ M). <sup>a</sup>
43	2,4-dichloro	<i>t</i> -butyl	b
49	2,4-dichloro	Methyl	66
50	2,4-dichloro	Phenyl	45
51	2,4-dichloro	Cyclohexyl	b
52	2,4-dichloro	Cyclopentyl	b
53	2,4-dichloro	Isobutyl	b
54	2,4-dichloro	Isovaleryl	b
44	3,5-dichloro	<i>t</i> -butyl	b
55	3,5-dichloro	Methyl	30
56	3,5-dichloro	Phenyl	27
57	3,5-dichloro	Cyclohexane	b
58	3,5-dichloro	Cyclopentane	b
59	3,5-dichloro	Isobutyl	b
60	3,5-dichloro	Isovaleryl	b
45	4-chloro	<i>t</i> -butyl	b
61	4-chloro	Methyl	31
62	4-chloro	Phenyl	13
45	4-trifluoromethyl	<i>t</i> -butyl	b
63	4-trifluoromethyl	Methyl	50
64	4-trifluoromethyl	Phenyl	38
65	4-trifluoromethyl	Cyclohexane	b
66	4-trifluoromethyl	Cyclopentane	b
67	4-trifluoromethyl	Isobutyl	b

<b>68</b>	4-trifluoromethyl	Isovaleryl	<sup>b</sup>
<b>47</b>	4-trifluoromethoxy	<i>t</i> -butyl	<sup>b</sup>
<b>69</b>	4-trifluoromethoxy	Methyl	44
<b>70</b>	4-trifluoromethoxy	Phenyl	34
<b>71</b>	4-trifluoromethoxy	Cyclohexane	<sup>b</sup>
<b>72</b>	4-trifluoromethoxy	Cyclopentane	<sup>b</sup>
<b>73</b>	4-trifluoromethoxy	Isobutyl	<sup>b</sup>
<b>74</b>	4-trifluoromethoxy	Isovaleryl	<sup>b</sup>

<sup>a</sup> Results obtained from the TLC assay. Mean of at least 2 measurements with typically a SD of  $\pm 5$  %,

<sup>b</sup> Not determined.

**Table 6.2:** Full range of novel amide linked target compounds synthesised and available enzyme inhibition data.

The limited biological data available on these compounds allow for some rudimentary conclusions regarding the effects of different substitutions on the diphenylether hydrophobic headgroup. Five different substitution patterns were tested and no dramatic differences between them were observed. All are viable substitution patterns for future investigations. It can be concluded, however, that the 2,4-diCl is preferred over 3,5-diCl substitution pattern, as the percentage inhibitions were obtained as 66 % and 27 % for the phenyl substituted compounds respectively (**49** and **56**). This is not conclusive as not all the compounds have been tested and the IC<sub>50</sub> values have not been obtained. In addition a 4 substituent is tolerated and 4-chloro, 4-CF<sub>3</sub> and 4-OCF<sub>3</sub> show moderate inhibition at 10  $\mu$ M. It is hoped that further biological data will give more detailed information regarding the effects of the substituents on the diphenylether hydrophobic headgroup.

There is one main pattern visible in this series, with the better inhibitory activities generally being shown by those compounds with a methyl group, rather than a phenyl group, in the hydrogen bond acceptor region of the template. The introduction of cyclohexane, cyclopentane, *t*-butyl, isobutyl and isovaleryl will help to explore the SAR surrounding the size and electronics in this region.

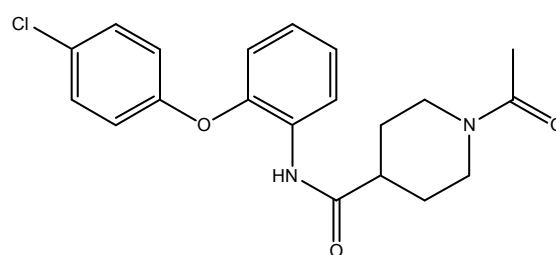
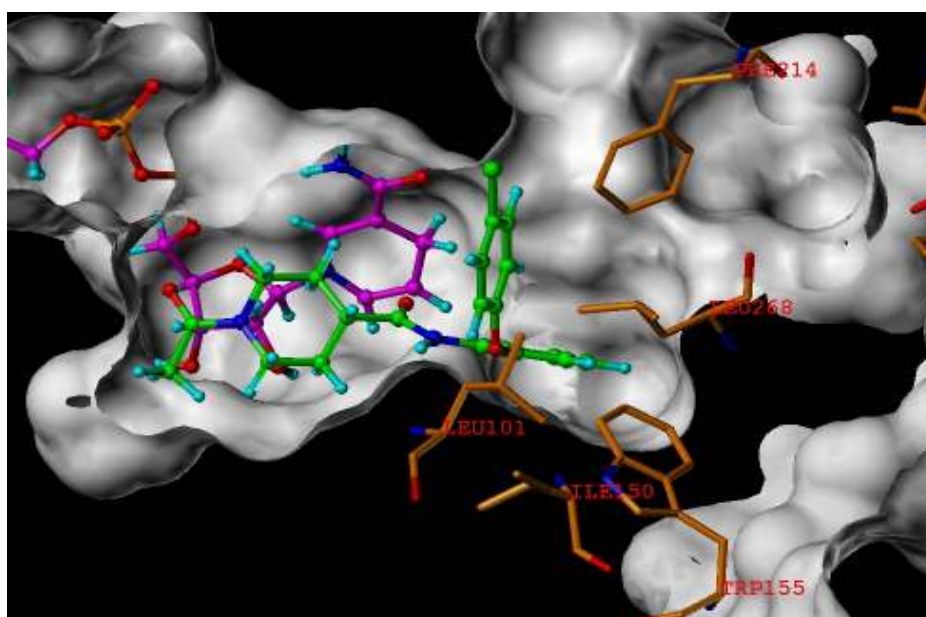
The difference between the methyl and phenyl substituted compounds may be simply due to the size difference between the two groups affecting the ability of the inhibitor to fit

effectively into the active site of the protein. Alternatively it may be due to electronic interactions or an induced change in the geometry/shape of the molecule. *In-silico* studies were able to propose an explanation for these differences.

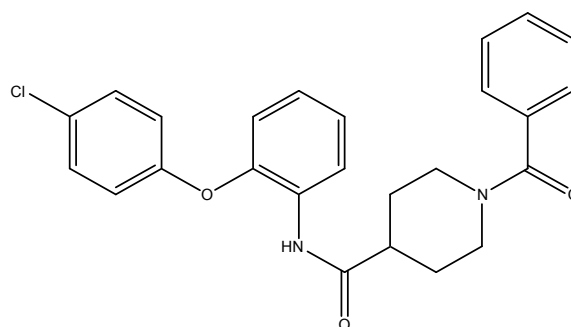
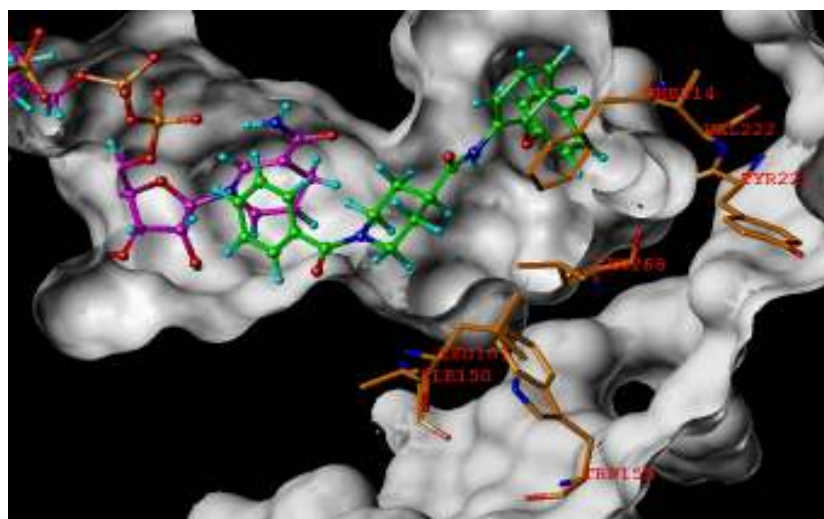
### 6.3 Docking Studies of the Amide Linked Series

All the compounds in Table 6.2 were docked into the 17 $\beta$ -HSD Type 3 homology model discussed in Chapter 4 by Dr A Smith and Dr M Trusselle. This presented some interesting observations that may help to explain the biological results. For example, compound **61** and compound **62** differ only in the R group in the hydrogen bond accepting region of the compound. Compound **62** has a phenyl ring, whereas compound **61** has the much smaller methyl group. Both have a 4-Cl substituted hydrophobic head group, linked via an amide bond to the piperidine ring. Figures 6.3 and 6.3 shows these 2 molecules docked into the 17 $\beta$ -HSD Type 3 homology model.

It can be seen that the two compounds sit very differently within the active site of the homology model. Compound **61** fits into the active site with the *N*-acetyl group towards the cofactor, possibly due to an interaction between the *N*-acetyl and the co-factor itself. This interaction seems to be absent in compound **62** and therefore the molecule is positioned completely differently. It is hard to know the exact reason for this, but it is possible that the phenyl ring is simply too large to allow the interaction between the amide and the co-factor to occur, thus indicating that the molecule can be attracted into a different region of the active site.



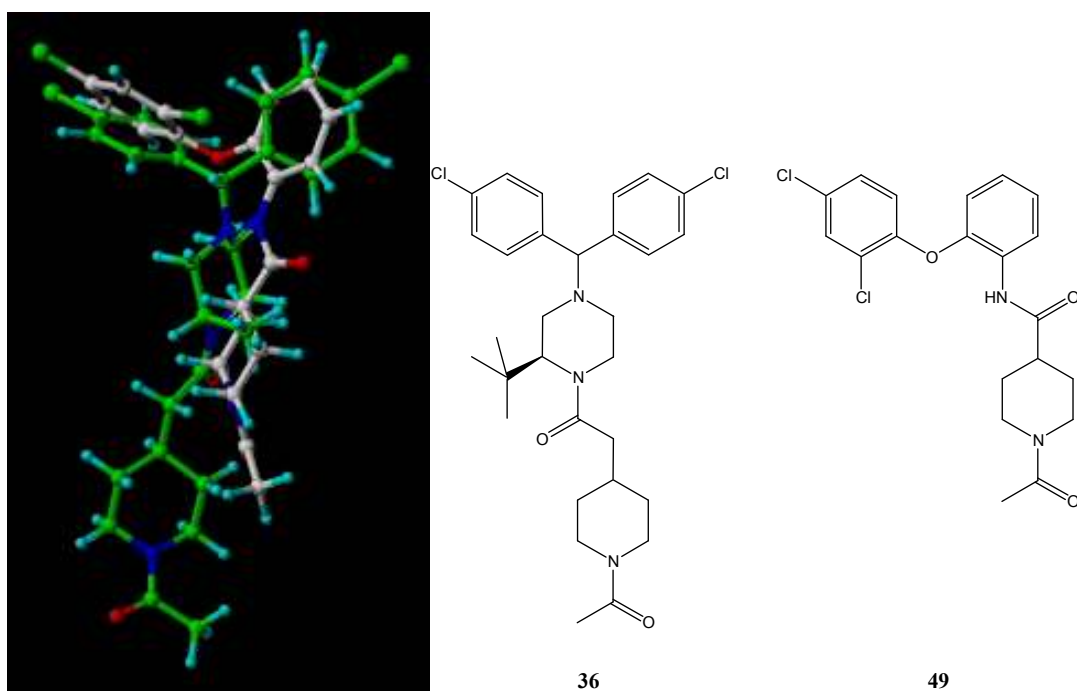
**Figure 6.3:** Compound **61** docked into the 17 $\beta$ -HSD Type 3 homology model.



**Figure 6.4:** Compound **62** docked into the 17 $\beta$ -HSD Type 3 homology model.

It is hypothesized that the active site of 17 $\beta$ -HSD Type 3 is spacious as there are many large reported inhibitors of the enzyme.<sup>28, 46</sup> This is supported by the docking studies, as space can be seen either above **61** or below **62**. It had been envisaged that the hydrophobic head group would fit into the large hydrophobic pocket of the active site, as can be seen with **61**. However, in **62** the molecule is not sitting in this region, as the distance between the headgroup and the *N*-acetyl group is too short. This may be the reason why the Schering-Plough positive control<sup>47</sup> (Chapter 2, **36**) exhibits a much higher potency than the compounds synthesised in this series so far. Compound **36** is much longer in length and therefore may be able to occupy both the hydrophobic pocket and interact with the cofactor simultaneously (Figure 6.5). It can be seen that there is a clear difference in size and length between compound **36** and the amide linked compound synthesised (**49**). For these reasons the synthesis of a new series of extended amide linked compounds was initiated.

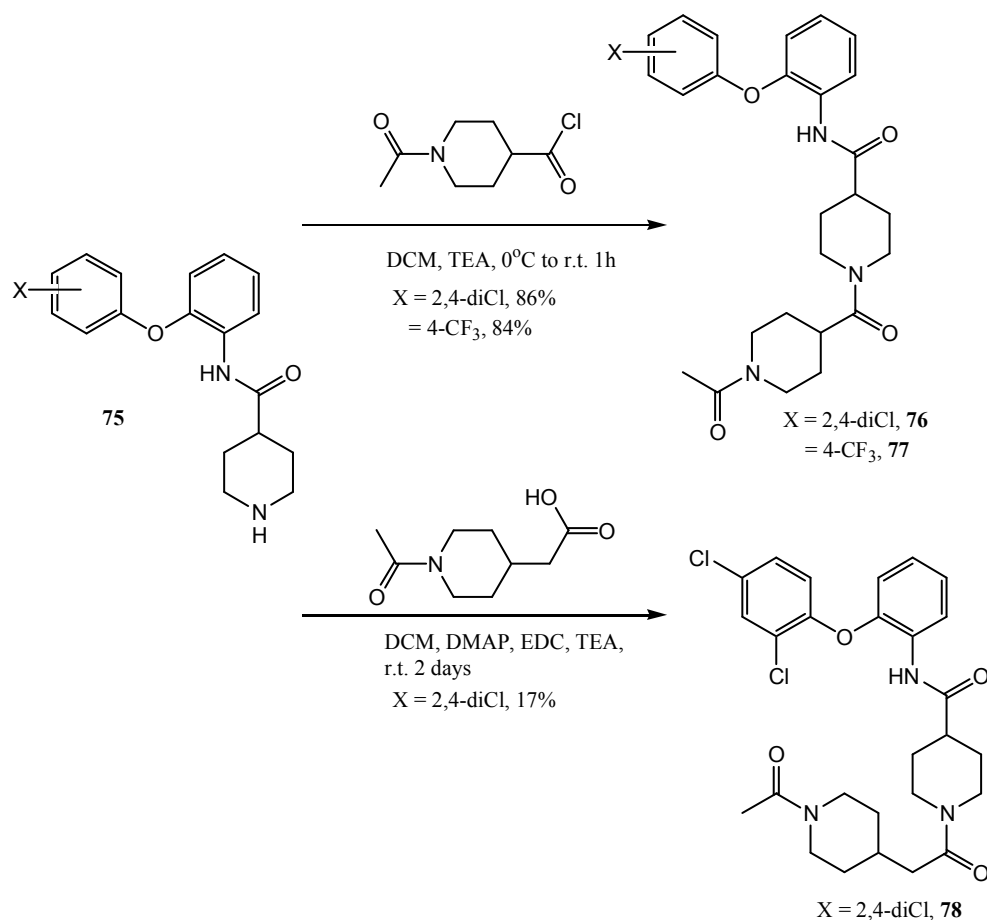




**Figure 6.5:** Comparison between **36** (Green) and **49** (White).

#### 6.4 Synthesis of Extended Amide Linked Compounds

The synthetic route to the extended amide linked compounds is shown in Scheme 6.2. The three compounds synthesised in this series, **76**, **77** and **78**, were all synthesised from the same intermediate (**75**) used to make the amide linked compounds described previously (Scheme 6.1). The synthetic route to the extended compounds is shown in Scheme 6.2. The piperidine amine **75** was reacted with 1-acetylpiperidine-4-carbonyl chloride, to form another amide bond linking the extra piperidine ring to the compound. Excellent yields of 86 % and 84 % were obtained for compound **76** and **77** respectively. The same piperidine amine **75** was also subjected to an amide coupling reaction with (1-acetyl-piperidin-4-yl)-acetic acid and although the yield of this reaction was low at just 17 %, the compound was synthesised successfully and submitted for biological testing.

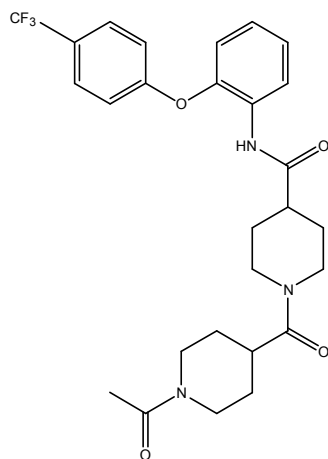
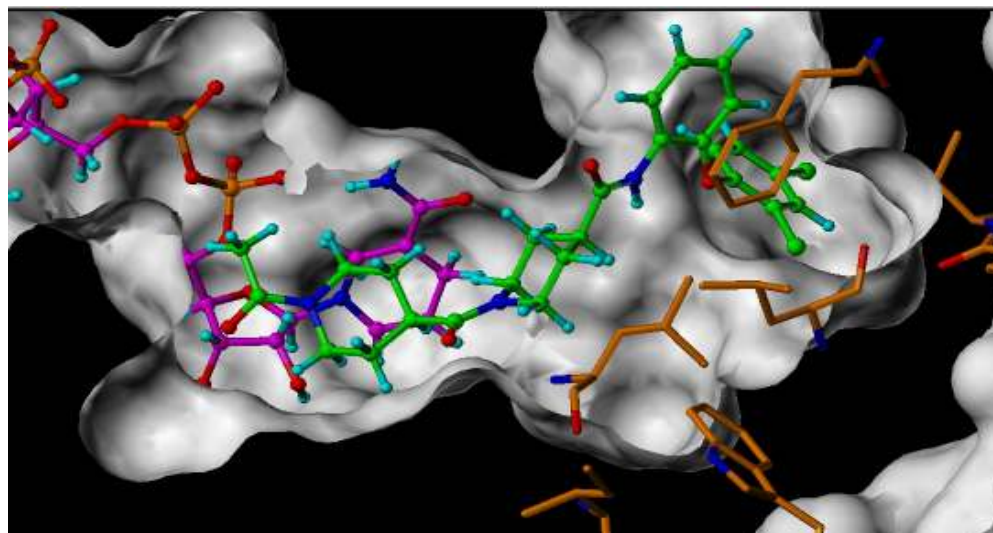


**Scheme 6.2:** Synthesis of extended amine linked compounds **76**, **77** and **78**.

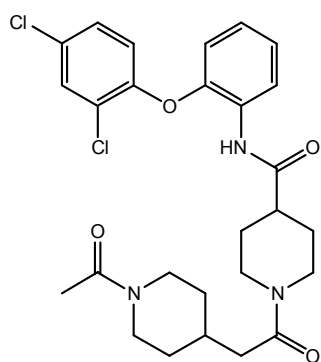
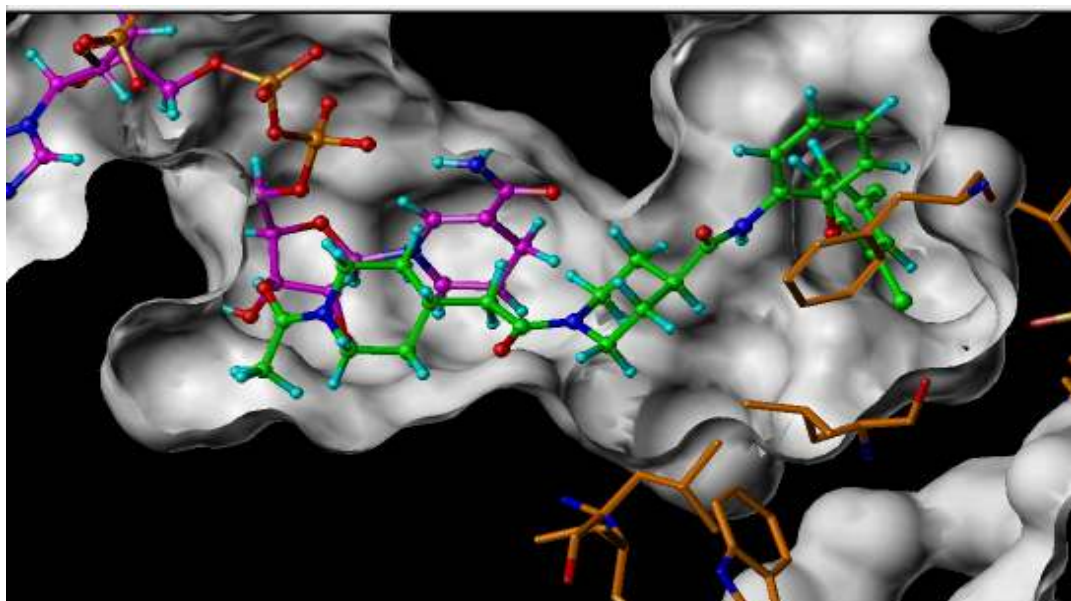
However, these compounds have not been subjected to biological testing so far, so no definite conclusions can be drawn from this series as yet. These compounds were docked into the 17 $\beta$ -HSD Type 3 homology model and this produced some interesting results.

The docking of the extended compounds **76** and **78** are shown in Figures 6.6 and 6.7. It can be seen from these docking studies that **76** and **78** both lie in a similar conformation within the active site of the homology model. The diphenylether headgroup of the molecules can be seen to be positioned deep within the large hydrophobic pocket of the active sites. This should help to increase the binding efficiency of the compounds and therefore the inhibitory activity. At the same time the increased length of these compounds may allow the *N*-acetyl group to interact with the cofactor, thus hopefully increasing the binding strength. It would appear that the presence of the extra CH<sub>2</sub> in **78** compared to **76** has little impact upon the way the compound is positioned within the active site.

The methyl group in the hydrogen bond accepting region of the two compounds is directed slightly differently. In **78** it is sitting in a small pocket within the active site, whereas in **78** this is not observed. This may or may not be significant to the activity. No firm conclusions can be reached until biological data has been obtained. From these docking studies it is anticipated that the three compounds in this series will exhibit enhanced inhibitory activity compared to the original, shorter amide linked compounds (**43** to **74**).

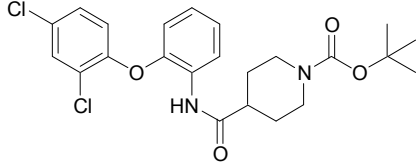
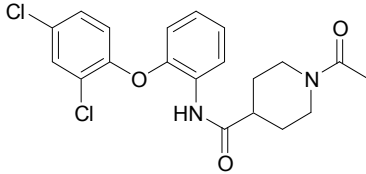
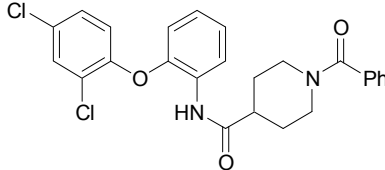
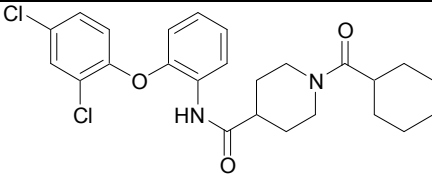
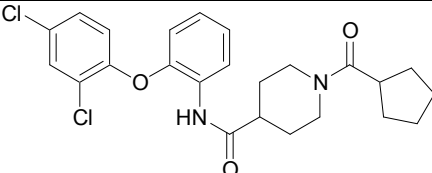
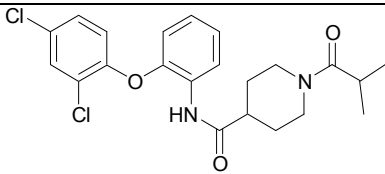
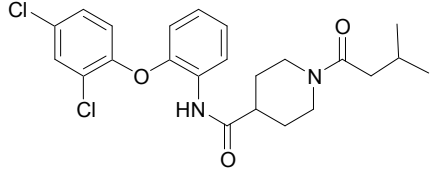


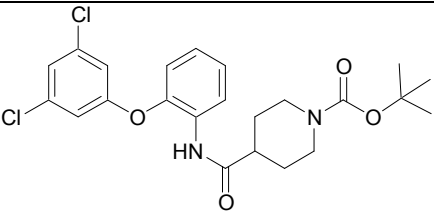
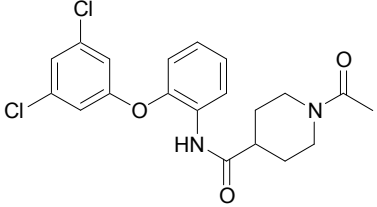
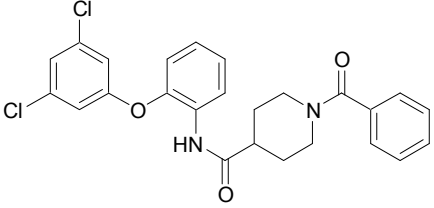
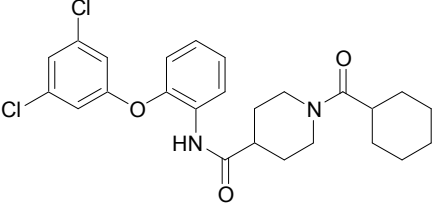
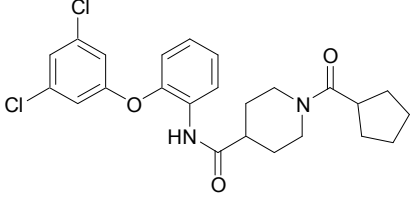
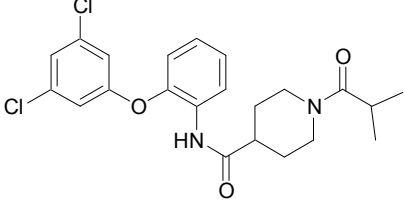
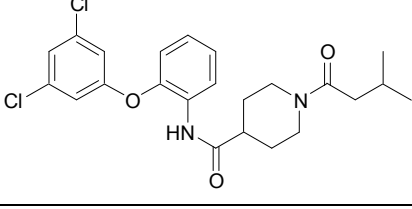
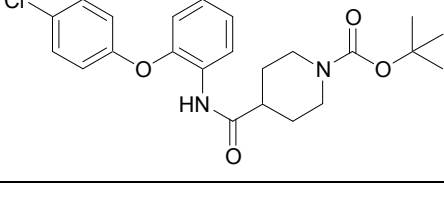
**Figure 6.6:** Compound **76** docked into the 17 $\beta$ -HSD Type 3 homology model.

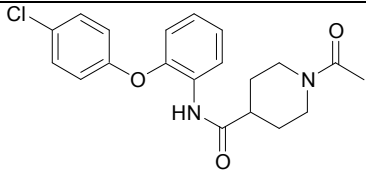
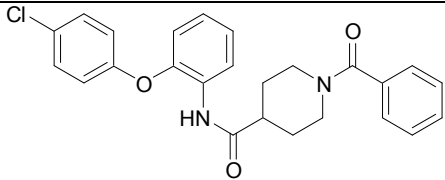
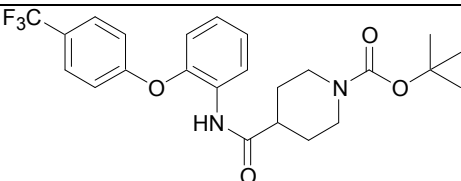
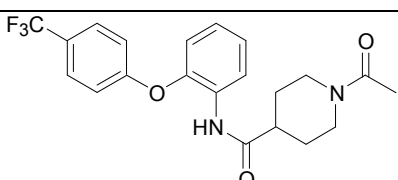
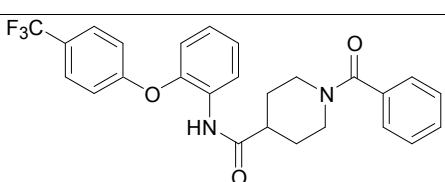
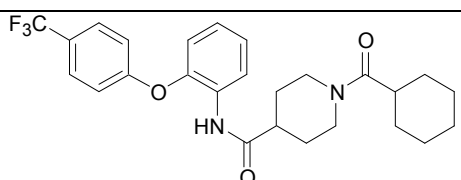
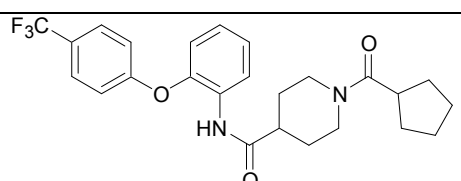
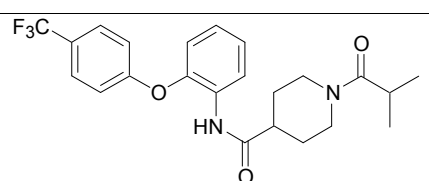
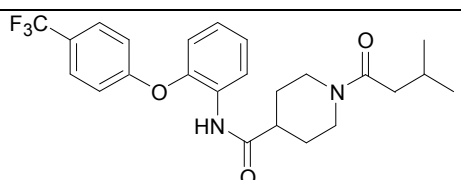


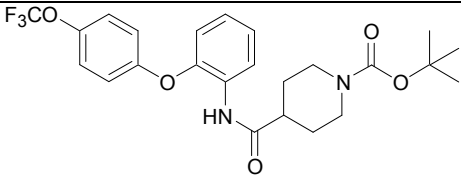
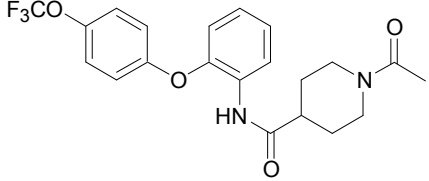
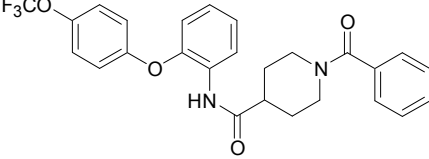
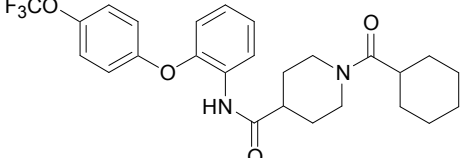
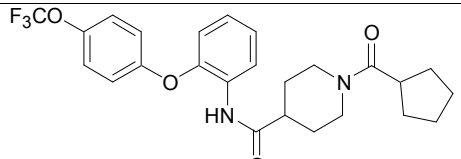
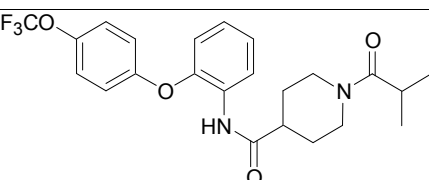
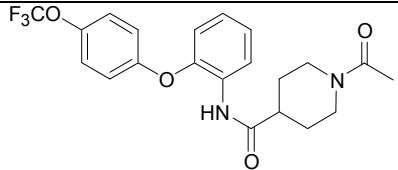
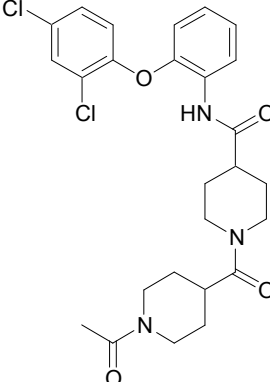
**Figure 6.7:** Compound **78** docked into the 17 $\beta$ -HSD Type 3 homology model.

## 6.5 Table of Compounds Synthesised within Chapter 6

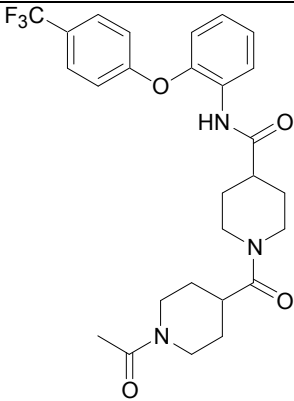
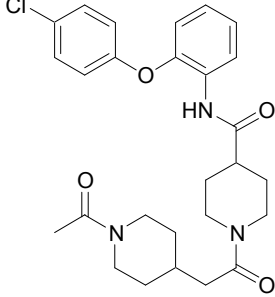
Compound no.	Structure	% Inhibition (at 10 $\mu$ M). <sup>a</sup>
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49		66
50		45
51		b
52		b
53		b
54		b

44		b
55		30
56		27
57		b
58		b
59		b
60		b
45		b

61		31
62		13
45		b
63		50
64		38
65		b
66		b
67		b
68		b

47		b
69		44
70		34
71		b
72		b
73		b
74		b
76		b



77		b
78		b

<sup>a</sup> Results obtained from the TLC assay. Mean of at least 2 measurements with typically a SD of  $\pm 5$  %,

<sup>b</sup> Not determined.

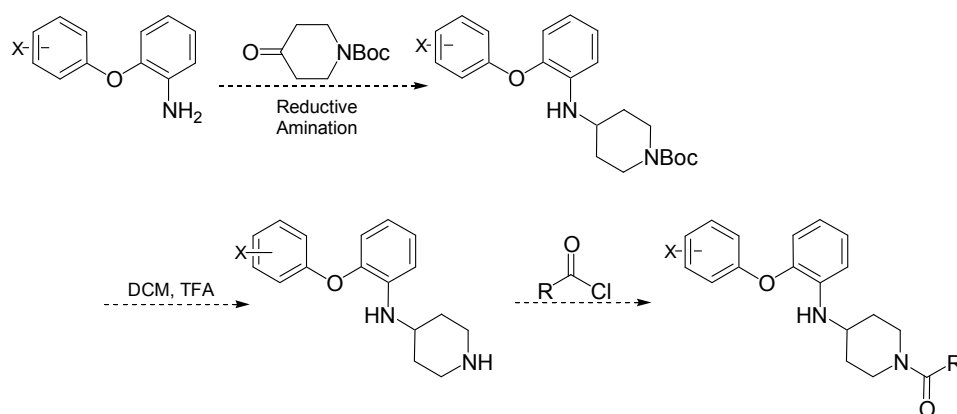
## Chapter 7

### Synthesis and Biological Evaluation of an Amine Linked Series of Inhibitors

In the initial stages of the project both amide and amine linked compounds were identified as potential inhibitors (Figure 5.4). The amide linked compounds were synthesised and showed some inhibitory activity. However, a large amount of investigation was still required to reach the potency level exhibited by our competitor compounds.<sup>46, 51, 52</sup> A series of novel amine linked compounds was therefore synthesised.

#### 7.1 Synthesis of Initial Targets

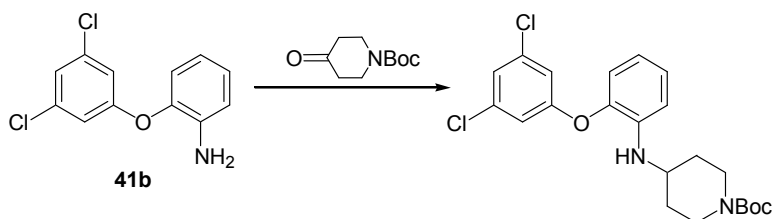
The initial amine linked target molecules were analysed retrosynthetically and their synthesis was planned and initiated (Scheme 7.1). The first two synthetic steps are common with the synthesis of the amide linked compounds. These common, diphenylether aniline intermediates were made in large batches and kept in stock for use in all series. The diphenylether aniline headgroup then required a reductive amination with *t*-butyl-4-oxo-1-piperidine carboxylate to form the amine bond. This reaction proved to be highly problematic.



**Scheme 7.1:** Proposed route to amine linked compounds.

The lower reactivity of anilines was considered before experimental procedures were initiated, and successful literature procedures were found, which detailed many similar reactions, using various different anilines and cyclohexanones.<sup>50, 67-71</sup> Many reductive amination methods were attempted (Table 7.1). These included direct reactions, where the piperidone and the amine were mixed with the reducing agent, without prior formation of

the imine (Table 7.1, Entries 2, 4, 5 and 6). Some methods were indirect/stepwise reactions, where the imine was preformed and was reduced in a second stage (Table 7.1, Entries 1 and 3). Overall, these attempted reductive amination methods met with limited success and a different method was required.



Entry	Reagents	Conditions	Yield (%)
1	<i>p</i> -TSA, 4 Å MS, toluene, Then NaBH <sub>4</sub> . <sup>71</sup>	Reflux 18 h r.t., 2 h	25
2	AcOH, THF, NaBH(OAc) <sub>3</sub> . <sup>50</sup>	r.t., 66 h	<sup>a</sup>
3	TiO <sup>i</sup> Pr, Then EtOH and NaCNBH <sub>3</sub> . <sup>67</sup>	r.t., 2 h r.t., 60 h	<sup>a</sup>
4	NiCl <sub>2</sub> ·6H <sub>2</sub> O, MeOH, NaBH <sub>4</sub> . <sup>68</sup>	50 °C, 5 h	<sup>a</sup>
5	Activated Zn dust, AcOH, H <sub>2</sub> O. <sup>70</sup>	70 °C, 5h	9
6	Activated SiO <sub>2</sub> , HCOOK, (CH <sub>3</sub> COO) <sub>2</sub> Pd, DMF. <sup>69</sup>	r.t., 18 h, 40 °C, 26 h	<sup>a</sup>

<sup>a</sup> No product isolated.

**Table 7.1:** Attempted Procedures for the Reductive Amination.

## 7.2 Development and Optimisation of a Novel Microwave Reductive Amination Procedure

Anilines are weakly basic amines, (pK<sub>a</sub> of 4.6, cf. 10.7 for cyclohexylamine<sup>72</sup>). This is partly due to a negative inductive effect from the nitrogen atom being attached to an sp<sup>2</sup> carbon but, more influentially it is caused by the partial delocalisation of the nitrogen lone pair into the aromatic ring. This reduced pK<sub>a</sub> means that anilines are less reactive than other primary amines.<sup>73</sup> This slows the initial attack on the carbonyl carbon and leads to a slower overall reaction rate.

Traditionally, sodium cyanoborohydride has been the reducing agent of choice in direct reactions.<sup>74, 75</sup> However, the reagent and its by-products are highly toxic, which is undesirable. Since then, sodium triacetoxyborohydride has been identified as a mild, selective and efficient alternative.<sup>76</sup> Imines are much more basic than ketones or aldehydes and thus are preferentially reduced by sodium triacetoxyborohydride.

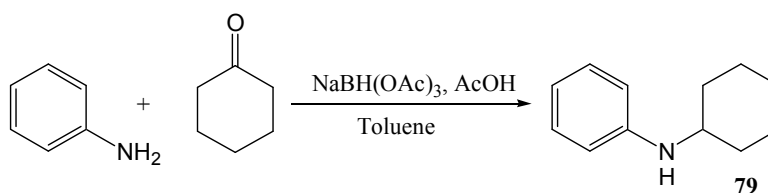
Due to the reactivity issues with anilines, Abdel-Magid's group demonstrated the use of several different sets of reaction conditions for the direct reductive amination of anilines using sodium triacetoxyborohydride.<sup>77-79</sup> Although their overall approach was successful, because of the numerous protocols reported, it seemed difficult to easily select optimal reaction conditions. It would therefore be useful if a more general, user-friendly protocol could be developed, thus allowing for the rapid synthesis of aromatic amines. It was also interesting to investigate whether the use of modern microwave technology could enhance the reaction rate and overall efficiency of the reductive amination process.

Since the pioneering work of Gedye<sup>80</sup> and Giguere,<sup>81</sup> the use of microwaves (MWs) in organic chemistry has become a quickly growing area of interest within synthetic organic chemistry.<sup>82-84</sup> Since the 1980s microwaves have been used in synthetic organic chemistry. However, it was not until the mid 1990s that the number of MW publications started to increase dramatically. The main reason for this was the availability of specialised commercial MW equipment intended for organic chemistry, which allowed for safer and more reliable, reproducible reactions.

Traditionally, most organic reactions are heated using heat transfer equipment, such as oil baths or heating mantles. However, these techniques can be rather slow and a temperature gradient can occur, which can lead to localised overheating, and the potential for decomposition or the formation of by-products. In contrast, MW radiation passes through the walls of the vessel and through dielectric heating, heats only the reactants and solvents. This helps to keep the temperature uniform throughout the vessel, which can lead to fewer by-products and/or decomposition and, therefore, often results in increased yields.<sup>84</sup> In pressurised systems, such as microwave reaction vessels, it is possible to heat solvents to a point far above the boiling point of the solvent, thus introducing a higher amount of energy to the reaction. New MW technologies are allowing investigations into large scale synthesis, batch processes, high-throughput library synthesis and flow processes. This could have a dramatic effect upon research and process chemistry.<sup>82-84</sup>

The principal advantage of the use of microwaves in organic chemistry is the dramatically reduced reaction times. As previously mentioned in Chapter 6, the formation of the diphenylether hydrophobic headgroup required heating at reflux for ~18 h, but this could be reduced to 30 min by utilising MW technology, without the yields being detrimentally affected. It was hoped that similar improvements could be made by using MW technology for the reductive amination of anilines.

At the outset of the study, and in order to develop and optimise a suitable microwave-assisted direct reductive amination process, the general method described by Abdel-Magid and co-workers was used as a basis.<sup>77-79</sup> A standard reaction between aniline and cyclohexanone was initially selected to carry out an initial time and temperature study (Scheme 7.2).<sup>85</sup> The reaction was carried out in sealed microwave tubes containing a solution of aniline (1 mmol), cyclohexanone (2 mmol), sodium triacetoxyborohydride (2.5 mmol) and acetic acid (3 mmol) in toluene (2 mL) which was then heated for various times and temperatures using a CEM Discover<sup>®</sup> instrument.<sup>66</sup>



**Scheme 7.2:** The reductive amination of cyclohexanone with aniline.

The results of the initial time and temperature study are shown in Table 7.2. Pleasingly, it was found that a smooth and successful reaction could be achieved at 100 °C in only 10 minutes (Entry 1). Moreover, it was discovered that the yields of desired product could be enhanced to a 87 % by increasing the temperature to 140 °C (Entry 3). Interestingly, heating beyond this temperature proved to be detrimental to the product yields (Entries 4 and 5). Furthermore, a 10 minute reaction time seemed to be optimal, as heating for a longer time period showed no significant benefits (Entries 6 and 7). Therefore, all further reactions were heated at 140 °C for 10 minutes.

Entry	Time (min)	Temperature (°C)	Yield (%) <sup>a</sup>
1	10	100	70
2	10	120	81
<b>3</b>	<b>10</b>	<b>140</b>	<b>87</b>
4	10	160	81
5	10	180	77
6	15	120	84
7	15	140	86

<sup>a</sup> Yield of isolated product.

**Table 7.2:** The effects of time and temperature on the reductive amination of cyclohexanone with aniline.

In order to further optimise the reductive amination process it was crucial to investigate an array of solvents that possess different microwave properties (Table 7.3).<sup>83, 86</sup> Whilst all solvents performed well, the yield of 94 % that was obtained with 1,2-dichloroethane was particularly impressive (Entry 2). It was therefore decided that all further reactions would be carried out using DCE as the solvent. Nevertheless, the result of 91 % obtained with acetonitrile (Entry 4) is comparable and, if required, this solvent could be used as an alternative.

Entry	Solvent	Yield (%) <sup>a</sup>
1	Toluene	87
2	THF	79
3	DCM	81
4	Acetonitrile	91
<b>5</b>	<b>1,2-DCE</b>	<b>94</b>

<sup>a</sup> Yield of isolated product.

**Table 7.3.** The effects of solvents used in reductive amination of cyclohexanone with aniline.

Investigations were also made to see whether the reducing agent or the acid could be successfully substituted for alternative reagents (Table 7.4). First, it was found that the use of both sodium cyanoborohydride and resin-bound MP-triacetoxyborohydride<sup>87</sup> resulted in inferior yields (Entries 1 and 2). However, the yield of ~70 % obtained with the use of the reducing agent MP-sodium triacetoxyborohydride (Entry 2) is still synthetically useful, as

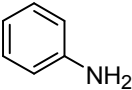
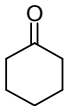
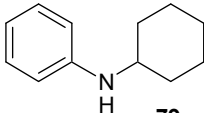
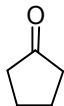
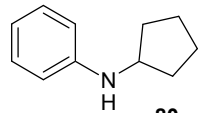
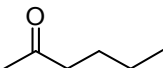
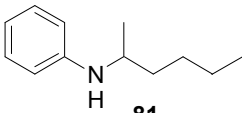
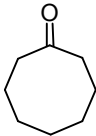
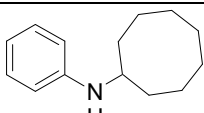
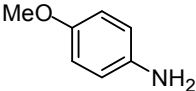
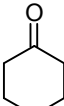
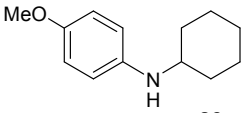
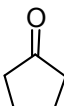
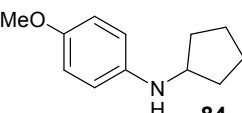
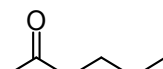
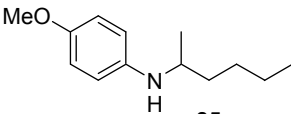
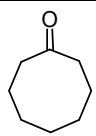
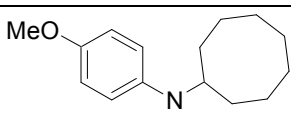
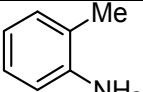
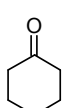
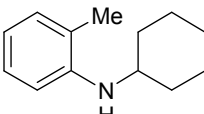
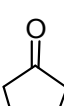
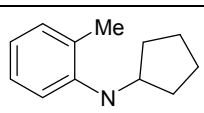
this reagent offers the benefit of a simple filtration rather than an aqueous work-up. The role of the acetic acid is clearly very important; it is thought to protonate the intermediate imine, therefore assisting in the reduction. All attempts to substitute this for other acid derivatives resulted in lower yields (Entries 3-5).

Entry	Acid	Reducing agent	Yield (%) <sup>a</sup>
1	AcOH	NaCNBH <sub>4</sub>	56
2	AcOH	MP-BH(OAc) <sub>3</sub> <sup>87</sup>	69
3	<i>p</i> -TSA	NaBH(OAc) <sub>3</sub>	45
4	Amberlite IRC50-H	NaBH(OAc) <sub>3</sub>	64
5	MP-TsOH	NaBH(OAc) <sub>3</sub>	35

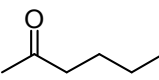
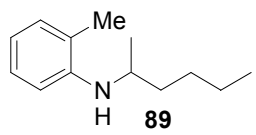
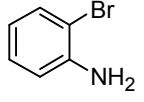
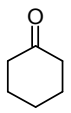
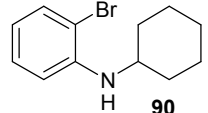
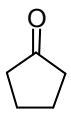
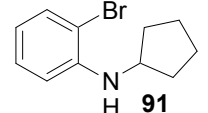
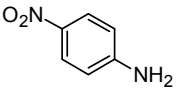
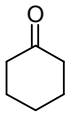
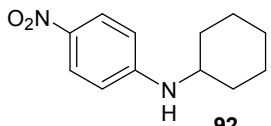
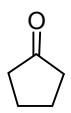
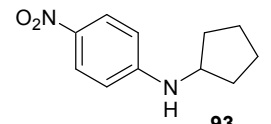
<sup>a</sup> Yield of isolated product.

**Table 7.4.** Alternative reagents for the reductive amination of cyclohexanone with aniline.

Now that optimised microwave conditions had been established,<sup>85</sup> reductive amination reactions were attempted with a range of electronically different anilines and ketones of varying reactivity (Table 7.5). Cyclopentanone and hexan-2-one reacted extremely readily with aniline to provide excellent yields of 88 % and 92 % respectively (Entries 2 and 3). Indeed, even the much less reactive cyclooctanone provided a very good yield of 68 % in only 10 minutes (Entry 4). In almost every instance the use of microwave irradiation to facilitate the reductive amination of various anilines was highly successful, with reactions reaching completion in just 10 minutes. Moreover, both *p*-methoxyaniline and *o*-toluidine performed extremely well with all the ketones used (Entries 5-11). However, and perhaps not surprisingly due to their lower reactivity, reactions involving the electron poor *o*-bromoaniline and *p*-nitroaniline gave lower yields (Entries 12-15). It should be noted that in these cases it was possible to observe some of the starting aniline by TLC. Nevertheless, this protocol still allowed for the swift formation of synthetically useful building blocks, albeit in moderate yields.<sup>85</sup>

Entry	Aniline	Ketone	Product	Yield (%) <sup>a</sup>
1	 (a)		 <b>79</b>	94
2	(a)		 <b>80</b>	88
3	(a)		 <b>81</b>	92
4	(a)		 <b>82</b>	68
5	 (b)		 <b>83</b>	77
6	(b)		 <b>84</b>	64
7	(b)		 <b>85</b>	87
8	(b)		 <b>86</b>	64
9	 (c)		 <b>87</b>	95
10	(c)		 <b>88</b>	81

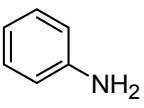
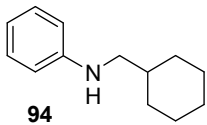
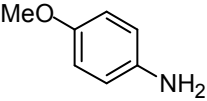
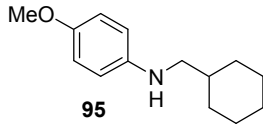
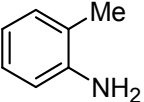
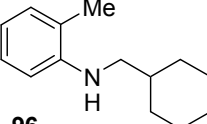
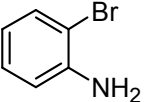
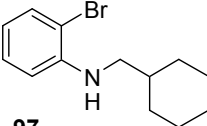
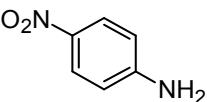
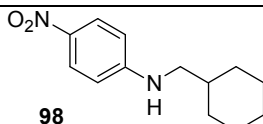


11	(c)		 89	92
12	 (d)		 90	56
13	(d)		 91	40
14	 (e)		 92	34
15	(e)		 93	26

<sup>a</sup> Yield of isolated product.

**Table 7.5.** Microwave-assisted reductive amination between anilines (a-e) and ketones.

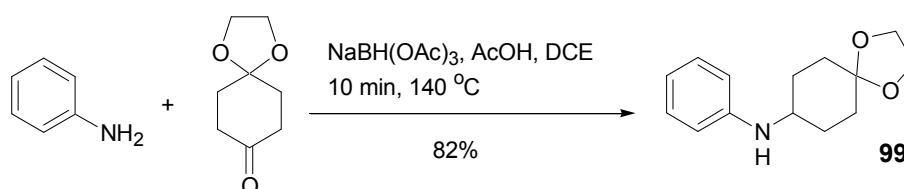
To fully explore the scope of these newly developed reductive amination conditions, the reaction between various anilines and an aldehyde, cyclohexanaldehyde, was examined (Table 7.6). However, due to the increased reactivity of aldehydes, when using the established protocol, the di-alkylated compound was found to be the major product. This problem was easily overcome by utilizing the aldehyde as the limiting reagent (2 mmol aniline with 1 mmol of aldehyde). Indeed, the desired mono-alkylated products were then obtained successfully in excellent yields of up to 91 %. Even the very un-reactive *o*-bromoaniline and *p*-nitroaniline provided the desired products in high yields of 75 % and 61 % respectively (Entries 4 and 5).<sup>85</sup>

Entry	Aniline	Product	Yield (%) <sup>a</sup>
1		 <b>94</b>	91
2		 <b>95</b>	83
3		 <b>96</b>	88
4		 <b>97</b>	75
5		 <b>98</b>	61

<sup>a</sup> Yield of isolated product.

**Table 7.6.** Microwave-assisted reductive amination between anilines and cyclohexanaldehyde.

Having established this novel microwave protocol, it was interesting to investigate whether acid labile groups were tolerated by the technique. To investigate this, 1,4-cyclohexanedione monoethylene acetal, which contains an acid labile acetal protecting group, was chosen to undergo a reductive amination with aniline (Scheme 7.3). Gratifyingly, using this general protocol the reaction proceeded extremely smoothly without cleavage of the acetal group, to afford the desired product with an excellent yield of 82 %.

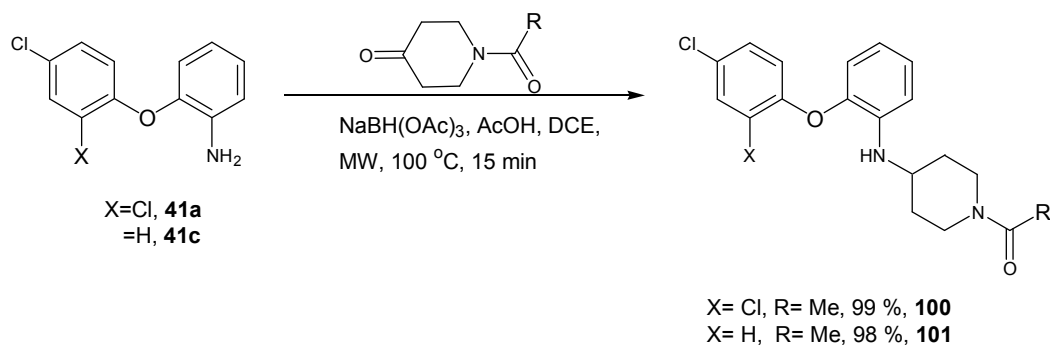


**Scheme 7.3:** Microwave-assisted reductive amination with an acid sensitive substrate.

This novel microwave-assisted direct reductive amination procedure offers many advantages over more traditional methods. The most beneficial is the rapid reaction time, with the vast majority of reactions producing excellent yields after merely 10 minutes. Additionally, the reaction conditions are mild and simple; sodium triacetoxyborohydride is much less toxic than the alternative sodium cyanoborohydride and acid sensitive functionality can be tolerated by this system. A further advantage of this procedure is that inert conditions are not necessary. This, coupled with only minimal work-up and straightforward flash chromatography, means that the reactions are operationally very simple.<sup>85</sup>

### 7.3 Application of Novel Microwave Method

When applied to the original amine linked 17 $\beta$ -HSD Type 3 target compounds, this new procedure led to greatly increased yields and much cleaner reactions (Scheme 7.4). It was also found that 1-acetyl-piperidin-4-one and 1-benzoyl-piperidin-4-one were commercially available; thus the initial synthetic route to methyl or phenyl substituted amides could be shortened by 2 steps, as the *N*-*t*-Boc deprotection and following acylation was no longer required. This revised route allowed for the successful synthesis of two of the final amine linked targets, compounds **100** and **101** (Scheme 7.4).

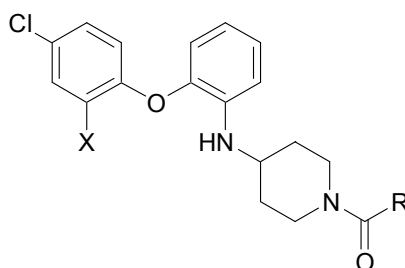


**Scheme 7.4:** Novel reductive amination procedure<sup>85</sup> to afford amine linked compounds **100** and **101**.

It should be noted that although the optimised conditions for reductive aminations with anilines were found to be 140 °C for 10 minutes, these are a guideline and excellent results can be obtained with alternative conditions. This means that the reaction can be successfully altered depending upon the substrates involved, for example compounds that are sensitive to high temperatures.

## 7.4 Biological Results and Discussion: Amine Linked Series

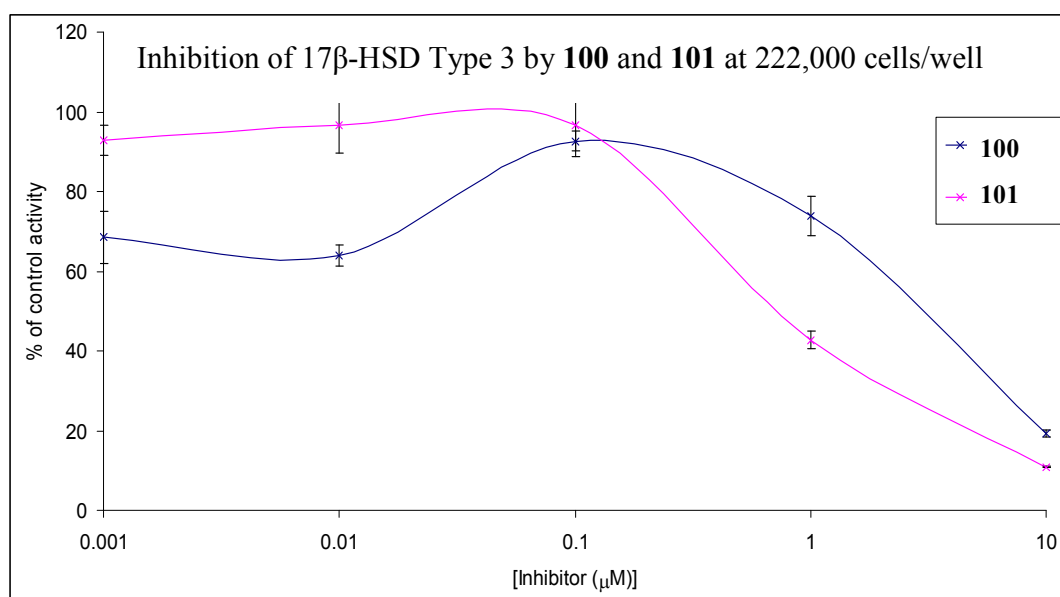
The methyl and phenyl derivatives of the amine linked target compounds were synthesised. These are detailed in Table 7.7. The IC<sub>50</sub> curves are shown in Figure 7.1.



Compound no.	X	R	% Inhibition at 10 $\mu\text{M}^{\text{a}}$	IC <sub>50</sub> ( $\mu\text{M}$ )
<b>100</b>	Cl	Me	84	2.7
<b>102</b>	Cl	Ph	64	<sup>b</sup>
<b>101</b>	H	Me	87	0.7
<b>103</b>	H	Ph	42	<sup>b</sup>
<b>104</b>	H	<sup>t</sup> Bu	43	<sup>b</sup>

<sup>a</sup> Results obtained from the TLC assay. Mean of at least 2 measurements with typically a SD of  $\pm 5\%$ , <sup>b</sup> Not determined.

**Table 7.7:** Biological results obtained for initial amine linked compounds.

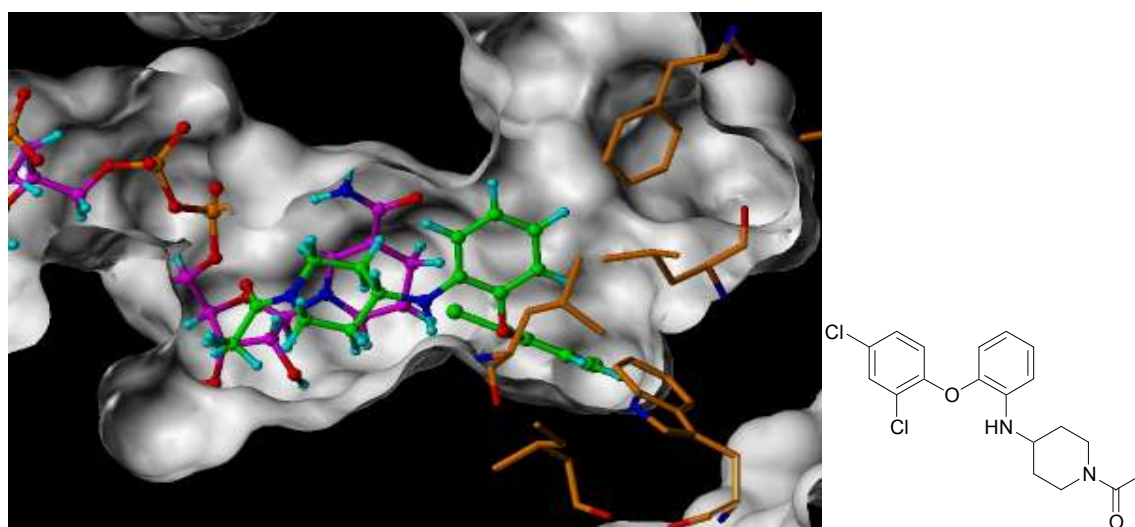


**Figure 7.1:** Curves to show the inhibition of 17 $\beta$ -HSD Type 3 by compounds **100** and **101**

It can be seen that those compounds containing a smaller R group, i.e. the methyl group in compounds **100** and **101**, show better inhibitory activity than those with the larger phenyl group, duplicating the pattern observed with the amide linked compounds. A *t*-Boc protected compound (**104**) was also submitted for biological testing and it can be seen that the *t*-Boc group is detrimental to activity (compound **104** compared to compound **101**). It can be observed that the presence/absence of chlorine at the 2-position of the diphenylether hydrophobic headgroup has only a small impact on the inhibitory activity of the compounds. The IC<sub>50</sub> values for compounds **100** and **101** have been measured. They are 2.7 and 0.7  $\mu$ M respectively. This shows that the amine linked compounds are a valid lead series and an excellent starting point for lead optimisation. A great deal of further investigation is required to fully develop the SAR of this series, surrounding both the diphenylether substituents and the size and electronics of the R group.

## 7.5 Docking Studies of the Amine Linked Compounds

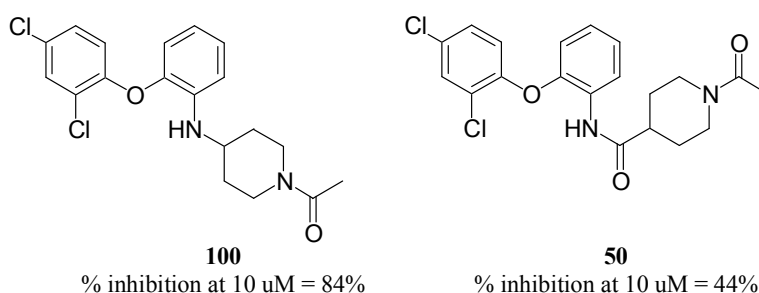
Figure 7.2 shows compound **107** docked into the 17 $\beta$ -HSD Type 3 homology model. It can be seen that this compound is positioned in a similar way to the previously mentioned amide linked compounds (Figures 6.2 and 6.3). The *N*-acetyl group of compound **107** is positioned towards the cofactor, with the diphenylether hydrophobic headgroup positioned towards a hydrophobic pocket. However, as with the original amide linked series, it appears that the compound is too short to be able to interact with both the cofactor and the hydrophobic region simultaneously. For this reason the design and synthesis of extended amine linked compounds may require attention at a later stage, dependent upon the inhibitory activity exhibited by the extended amine linked compounds.



**Figure 7.2:** Compound **100** docked into the 17 $\beta$ -HSD Type 3 homology model.

## 7.6 Comparison of the Amide and Amine Linked Series of Compounds

Even from the limited amount of biological data available, it is possible to determine a distinct pattern emerging in comparison of the two main series so far. The amide linked compounds show reduced activity in comparison with the corresponding amine linked derivatives previously described. For example, **100** and **50** (Figure 7.3) have percentage inhibitions of 84 % and 44 % respectively.



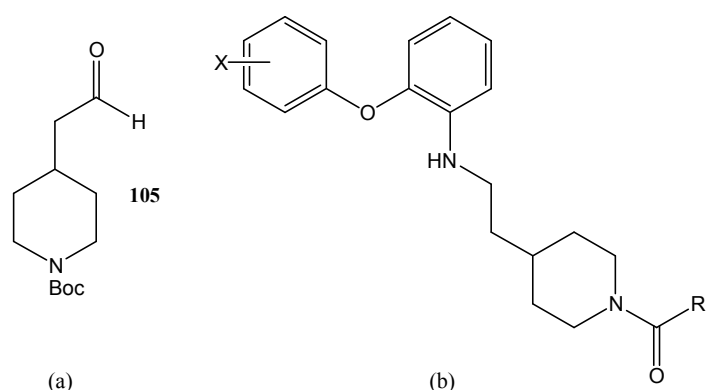
**Figure 7.3:** Comparison between amine (**100**) and amide (**50**) linked target compounds.

There are no clear cut differences in activity between the various hydrophobic head group substitutions tested in the series so far, indicating they are all valid templates, as implied by the Schering-Plough patents,<sup>47-50</sup> although 4-chloro and 2,4-dichloro do show a slight improvement in activity over the other substitutions. From all the biological results obtained so far, it can be concluded that the most promising compounds are those that have a 4-substituted hydrophobic head group and an amine linking group.

For the next areas of investigation, the 2,4-dichloro head group and amine linker were kept constant, to allow effective comparisons between compounds during the SAR exploration.

## 7.7 Synthesis of Alternative, Flexible Extended Amine Linked Compounds

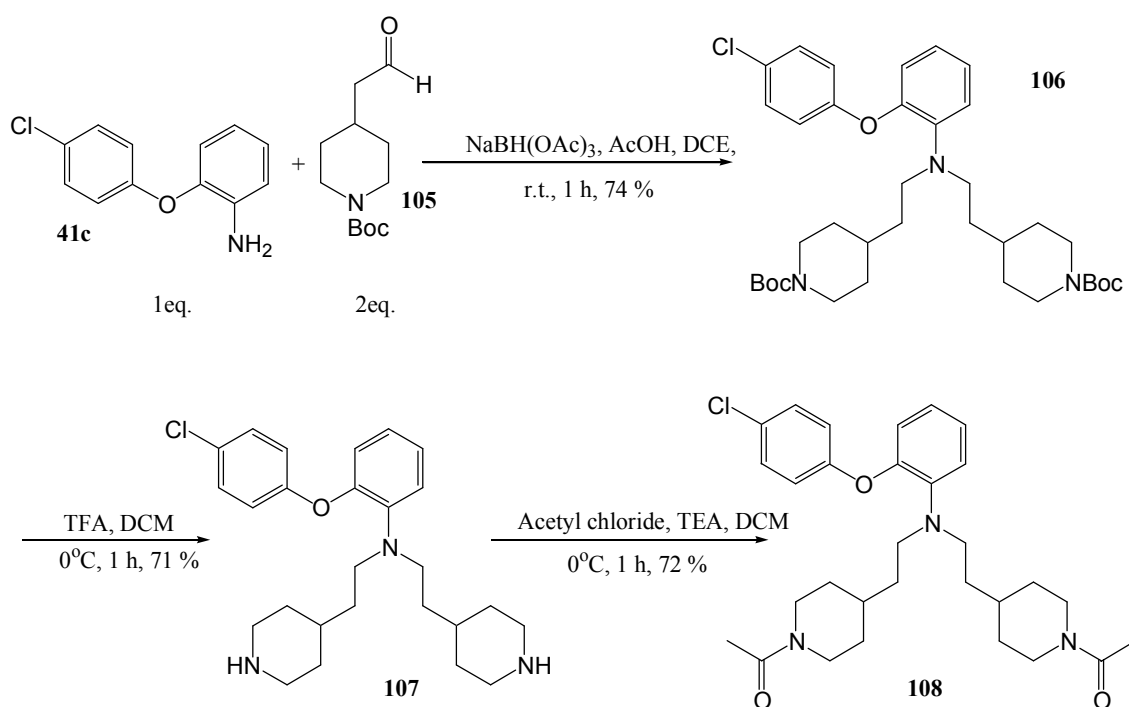
New reagents and synthetic building blocks are constantly being developed and launched by the various chemical suppliers. During the course of the project, a new product was launched by SigmaAldrich: *N*-Boc-4-piperidine acetaldehyde **105** (Figure 7.4a).<sup>88</sup> This could be used to make a novel alternative extended amine linked series of compounds, (Figure 7.4b).



**Figure 7.4:** (a) *N*-Boc-4-piperidine acetylaldehyde (**105**), (b) Initial targets in this series.

These compounds would be an extension of the original amine linked targets, which appear to be too short for optimal activity. It is hoped that the introduction of this novel two-carbon amine linking group may help to make the compounds more active, as the length may be more optimal. This linking group is also highly flexible, which may help the compound form a favourable conformation within the enzyme.

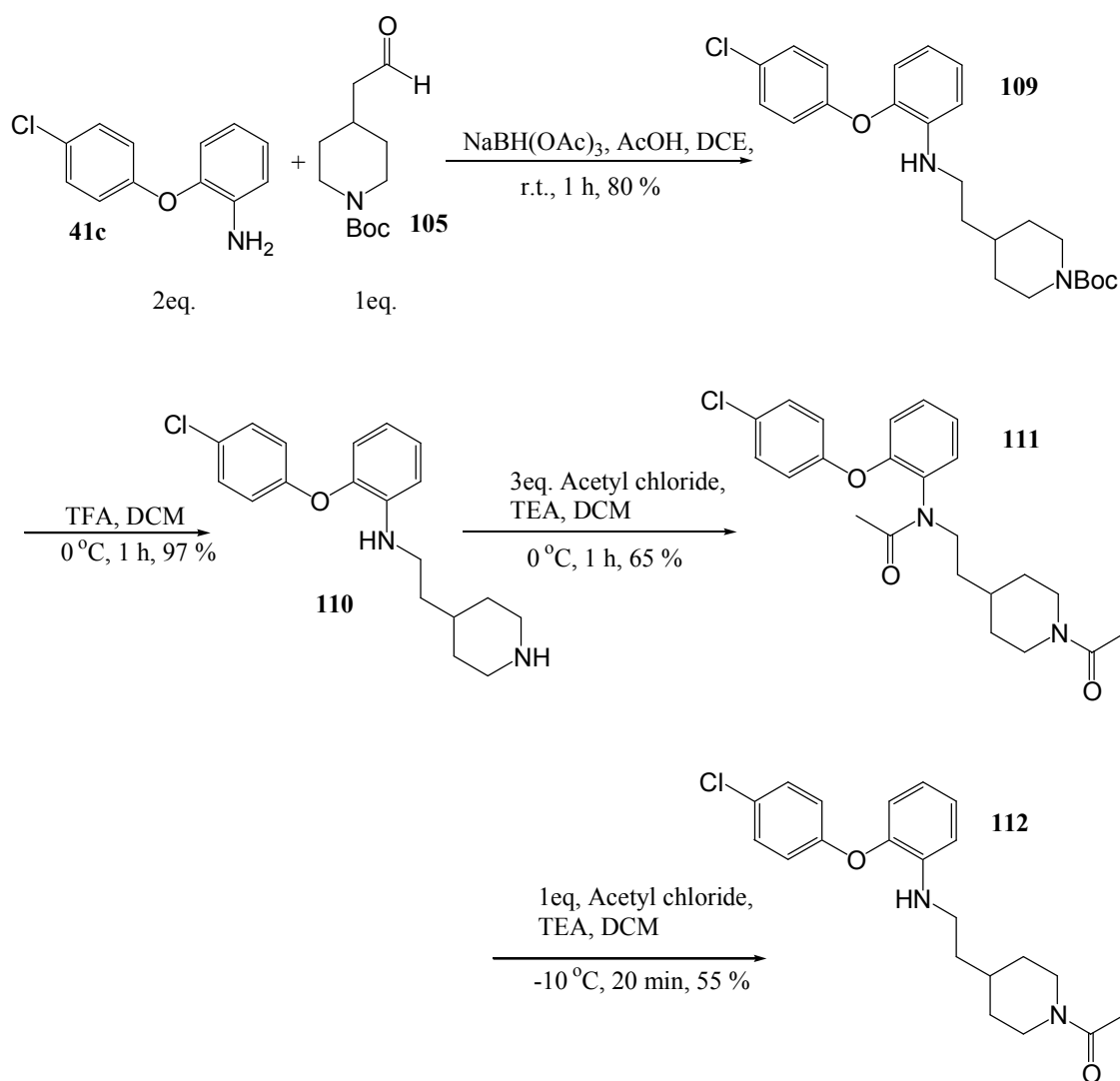
The synthesis of the target was initiated (Scheme 7.5). However, a mistake was made in the first step synthetic step. In this reductive amination procedure, two equivalents of the aldehyde were used and due to the increased reactivity of aldehydes compared to ketones, the reaction produced the di-substituted compound (**106**). This was deprotected and acetylated as normal, to produce 2 compounds in this series (**106** and **108**). It is not known whether these compounds exhibit any inhibitory activity over 17 $\beta$ -HSD Type 3. However, as previously mentioned the active site of 17 $\beta$ -HSD Type 3 is very large and these compounds will be useful to investigate the size tolerability of the enzyme.



**Scheme 7.5:** Synthesis of alternative di-substituted extended amine linked compounds, **106** and **108**.

The synthetic route was repeated using the correct ratio of starting materials. This led to the desired intermediate (**109**, Scheme 7.6). This was deprotected successfully using the standard conditions, leaving just the acetylation required to lead to the correct product. Using the standard acetylation conditions (3 eq. acetyl chloride, 3 eq. TEA, 0 °C to r.t. 1 h), the desired product was not obtained, but an alternative **111** was formed, where the piperidine nitrogen and the aniline nitrogen were both acetylated. This constitutes an interesting compound in itself, as it may help to understand the effects of the NH moiety upon activity. Alteration of the acetylation conditions (1 eq, acetyl chloride, 1 eq TEA, -10 °C for 20 min) led to the desired product (**112**) in 55 % yield.





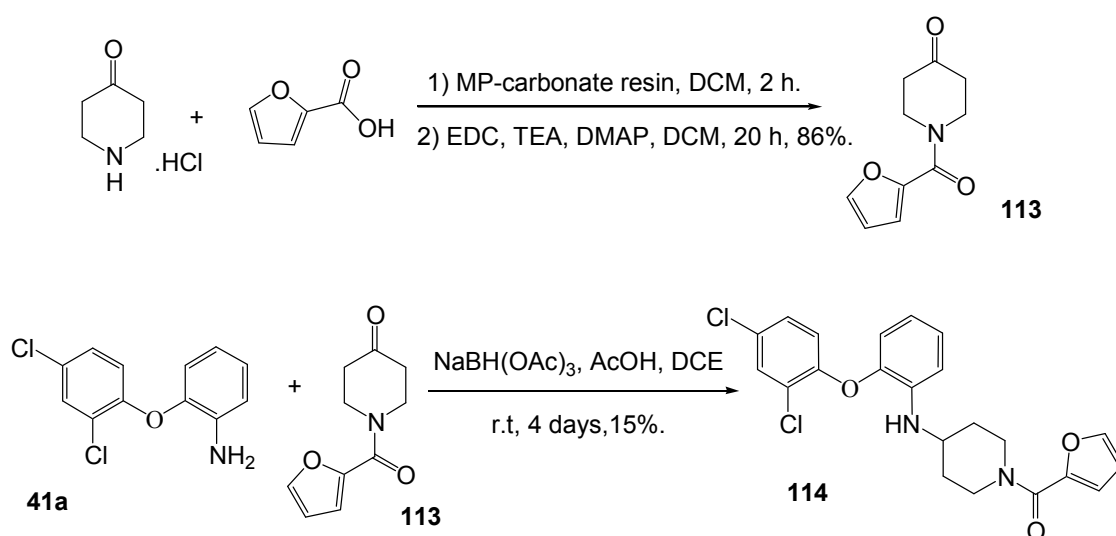
**Scheme 7.6:** Synthesis of alternative extended amine linked compounds **109**, **111** and **112**.

In summary, five compounds have been synthesised in this series, (including the *t*-Boc protected intermediates): two di-substituted compounds (**106** and **102**), and three mono-substituted compounds (**109**, **111** and **112**). These five compounds have been submitted for biological testing, and the results should give much information regarding size, shape and electronics of the active site.

## 7.8 Introduction of Heterocyclic and Adamantyl Groups on to the Piperidine Ring

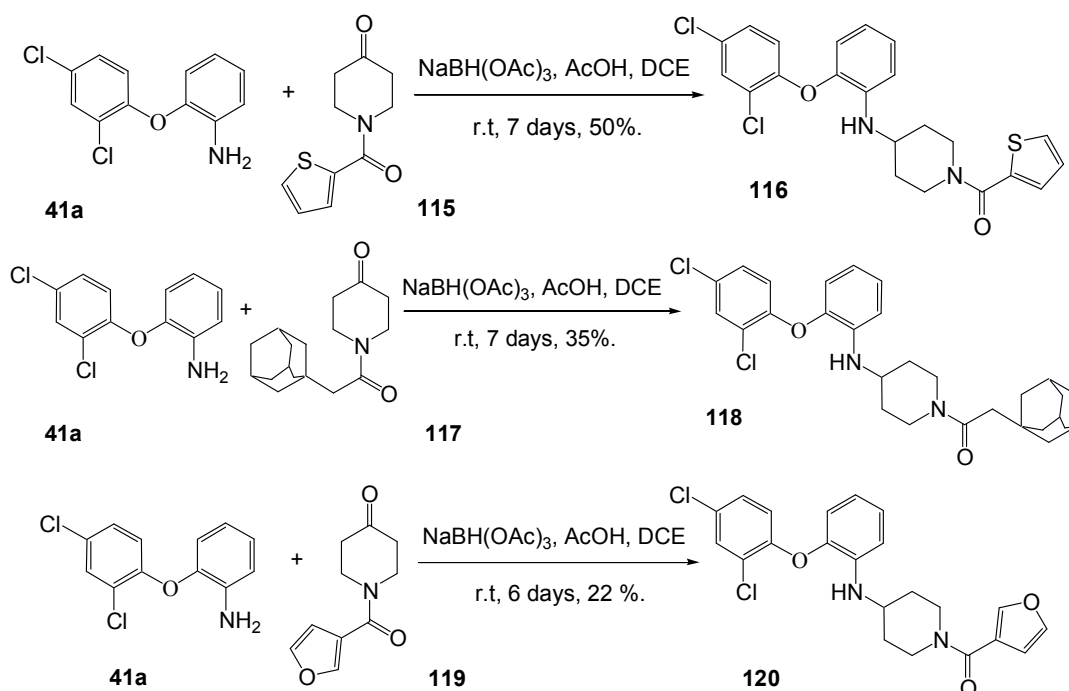
To further investigate the SAR of the R group substitution in the hydrogen bond acceptor region of the template, it was decided that heterocycles and large hydrophobic cyclic structures should be introduced. These are much larger groups, which have differing electronics to those compounds already made. The new R groups were to be incorporated into one of the most successful series so far, the 2, 4-dichloro substituted diphenylether head with an amine linking group. The planned cyclic moieties included thiophene, furan, adamantyl and pyridine groups.

The first to be synthesised was the furan derivative **114** (Scheme 7.7). The first synthetic step was the amide coupling of 2-furoic acid with piperidin-4-one. Piperidin-4-one was only available as the hydrochloride salt, and the free amine was required before amide coupling could occur. The free amine was released using a resin bound equivalent of triethylammonium carbonate (MP-carbonate resin).<sup>87, 89</sup> To ensure that the amine was free to react with the desired activated carboxylic acid, the resin remained in the reaction throughout the reaction duration. A standard amide coupling method, using EDC to activate the carboxylic acid, afforded compound **113** with an excellent yield of 86 %. The use of HOBt is known to decrease racemization but, more importantly in this case, speed up amide couplings.<sup>73</sup> However, no significant reduction in reaction time was observed when HOBt was utilised in this system. The piperidone **113** was then reacted with the diphenylether aniline, **41a**. Initially, the reductive amination was attempted using the previously established microwave procedure.<sup>85</sup> In this case, the conditions led to the decomposition of starting materials, and no product was obtained. This may be due to the promotion of side reactions. For example, a Diels-Alder reaction with the furan ring. The reductive amination was then attempted at room temperature and, although a greatly increased reaction time was used (~ 4 days), compound **114** was synthesised in just 15 % yield (Scheme 7.7). This synthetic procedure is far from optimised and further exploration of the conditions would be required before a large series of further compounds could be synthesised.



**Scheme 7.7:** The synthetic route to **114**

Once this general route had been initially developed, it was applied to the synthesis of the other desired cyclic structures, 3-furan, adamantyl and thiophene (Scheme 7.8). The piperidin-4-one hydrochloride was reacted with the desired acid in an amide coupling reaction. The reactions took ~18 h and gave the desired products in moderate yields of between 43-63 %. As with the synthesis of the 2-furan substituted compound, extensive reaction times and low yields (between 22 % and 50 %) were observed for the reductive amination.

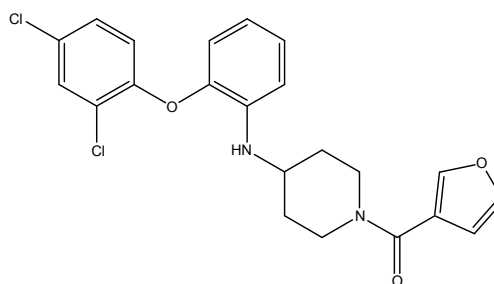
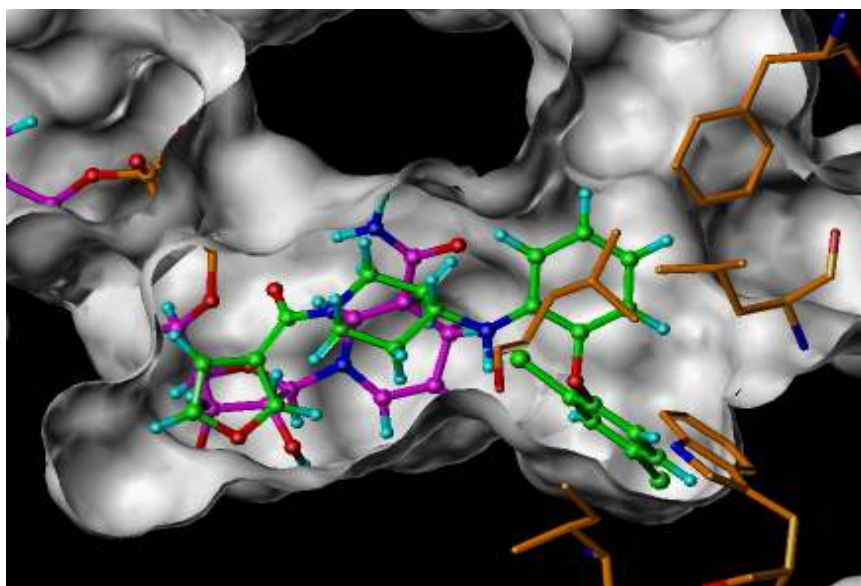


**Scheme 7.8:** Preparation of further compounds with cyclic R groups.

Unfortunately, the reductive amination proved to be unsuccessful in the attempted synthesis of the pyridine analogue, 4-[2-(2,4-dichloro-phenoxy)-phenylamino]-piperidin-1-yl-pyridin-3-yl-methanone, and only the starting aniline was recovered. This may be due to the basic nitrogen in the pyridyl group interfering with the desired reaction.

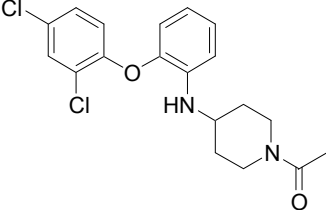
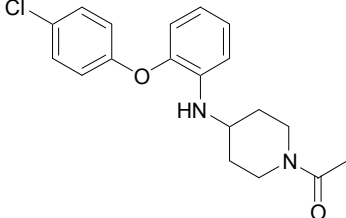
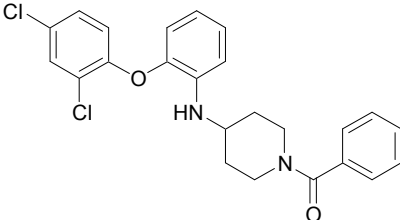
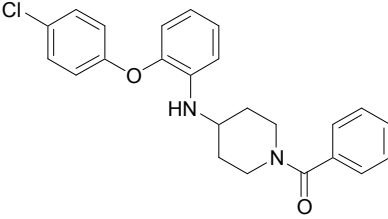
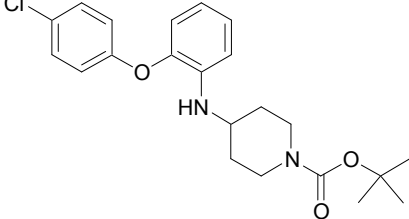
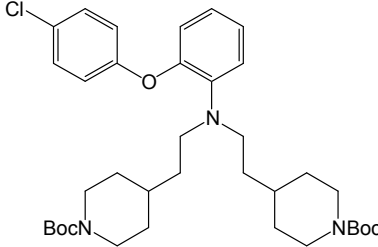
In conclusion, 4 compounds in this series have been synthesised (**114**, **116**, **118** and **120**), purified to >95 % purity and fully characterised. These compounds have been submitted for biological evaluation, but have not undergone testing yet.

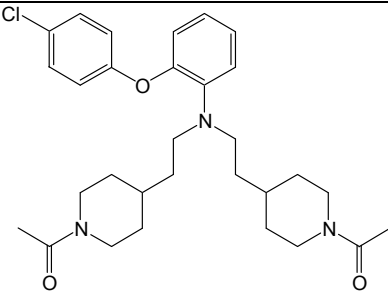
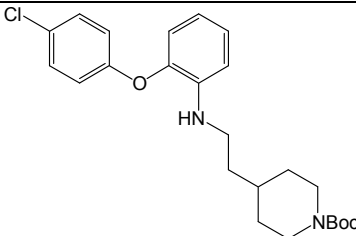
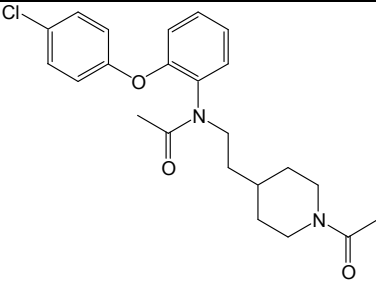
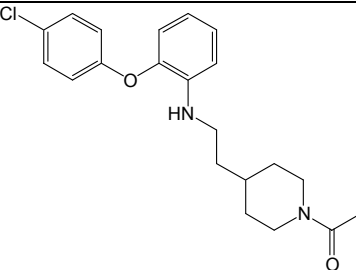
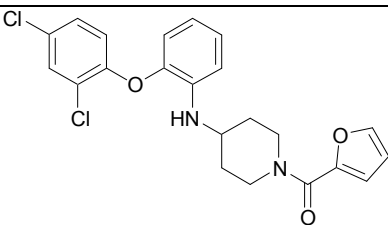
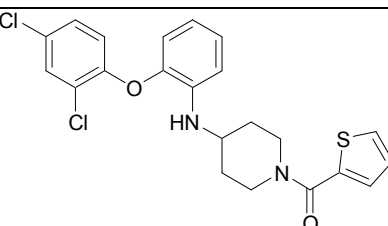
These compounds have been docked into the 17 $\beta$ -HSD Type 3 homology model. Figure 7.5 shows compound **120**, the 3-furan substituted compound. It can be seen that this compound is positioned in a similar manner to **100**, with the carbonyl at the base of the molecule interacting with the cofactor, and the hydrophobic head group directed towards the hydrophobic pocket of the active site. It is also possible that the oxygen in the furan ring may interact with the cofactor, but it is not known whether this will be beneficial or otherwise to the binding and inhibitory activity of the compound. Until biological results are obtained it cannot be deduced how the introduction of the furan ring, and the other heterocycles and large hydrophobic cyclic substitutions will affect the inhibitory activity of the compounds.

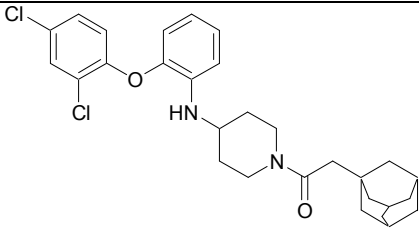
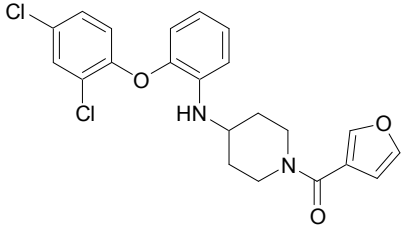


**Figure 7.5:** Compound **120** docked into the 17 $\beta$ -HSD Type 3 homology model.

## 7.9 Table of Compounds Synthesised within Chapter 7

Compound no.	Structure	% Inhibition (at 10 $\mu$ M). <sup>a</sup>
100		84
101		87
102		64
103		42
104		43
106		b

108		b
109		b
111		b
112		b
114		b
116		b

<b>118</b>		b
<b>120</b>		b

<sup>a</sup> Results obtained from the TLC assay. Mean of at least 2 measurements with typically a SD of  $\pm 5\%$ ,

<sup>b</sup> Not determined.

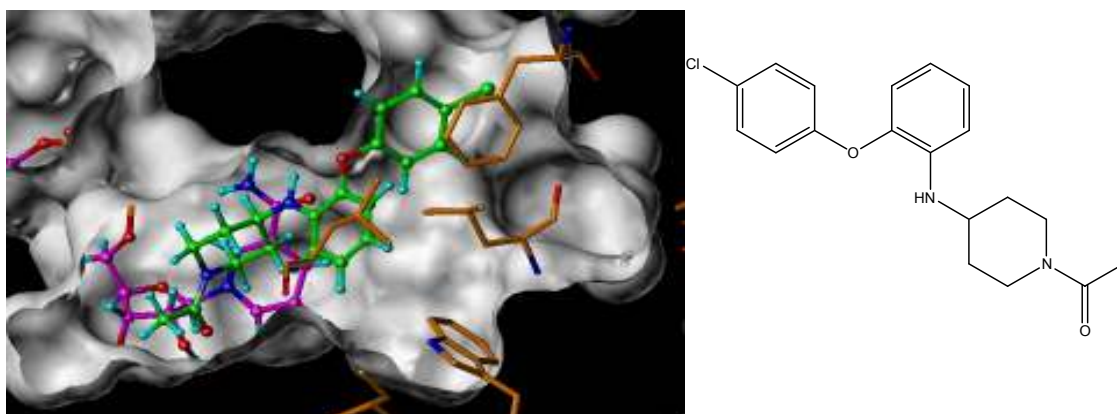


## Chapter 8

### Design and Synthesis of Benzylamine Linked 17 $\beta$ -HSD3 Inhibitors

#### 8.1 Introduction

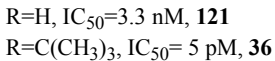
So far the best activity in this project has been exhibited by **101** (Table 7.7), with an IC<sub>50</sub> of 0.7  $\mu$ M. This represents an excellent lead compound, but there is still great scope for improvement. The homology model of 17 $\beta$ -HSD Type 3 has been very useful to the project. It has enabled some conclusions to be made about the size and shape of the active site. The active site is large and therefore it is anticipated that the enzyme will tolerate compounds that are much larger, either in length or bulk (see Chapters 6 and 7). It can be seen from the docking of **101** (Figure 8.1), that there is much unoccupied space around the inhibitor, especially around the central piperidine ring and the hydrophobic head group. Therefore, there is scope for further substitution and alternative flexible linkers to hopefully facilitate additional interactions in the active site.



**Figure 8.1:** Compound **101** docked into the 17 $\beta$ -HSD Type 3 Homology Model.

Increasing the length of the molecule (see Chapters 6 and 7) may help to realign the compound, so that it can interact with other hydrophobic regions and the cofactor, but this still appears to leave a large amount of space around the central piperidine ring. The Schering-Plough competitor compounds, such as compound **36** (see Chapter 5) also help to illustrate the importance of this region. A key feature of the Schering-Plough compounds is the large hydrophobic region (e.g. *t*-butyl) on the central piperidine ring. The importance of this region was demonstrated (Figure 8.2), the activity greatly increases with the

pM, a greater than 600 fold potency increase.<sup>46</sup>

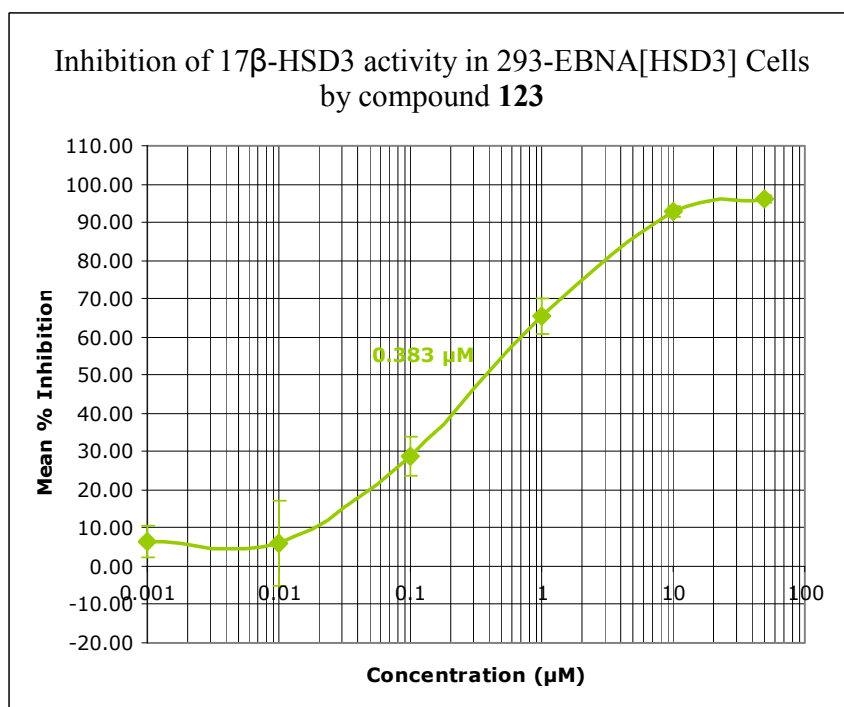


**Figure 8.2:** Improvements shown following the inclusion of a *t*-butyl group in the Schering-Plough competitor compounds.<sup>46</sup>

It was, therefore, very important that this region of the molecule was investigated further. The synthesis of compounds **122** and **123** was carried out by Dr C Sharland. Initially, this region was explored by introducing a large aromatic substituent (Figure 8.3) which actually led to a reduction in inhibitory activity, with an IC<sub>50</sub> for compound (**122**, Figure 8.3) of 28 µM. Although a reduction in activity was observed, some level of activity remained, thus showing that enlargements are tolerated, and that there is therefore potential around this region. When a seven-membered ring was introduced (**123**, Figures 8.3 and 8.4), the activity of this series was dramatically improved with an IC<sub>50</sub> of 383 nM.



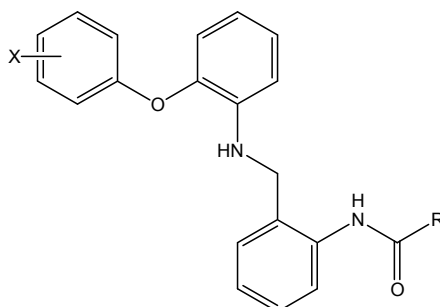
**Figure 8.3:** Benzofused *N*-acetyl piperidine (**122**) and azopine linked (**123**) compounds.



**Figure 8.4:** Inhibition of 17 $\beta$ -HSD Type 3 by compound **123**

This implies that there are spatial restrictions in this region of the active site, and that exploiting this space is likely to be very beneficial for activity, although it is possible that some restrictions will lead to lower activities. These need to be fully explored.

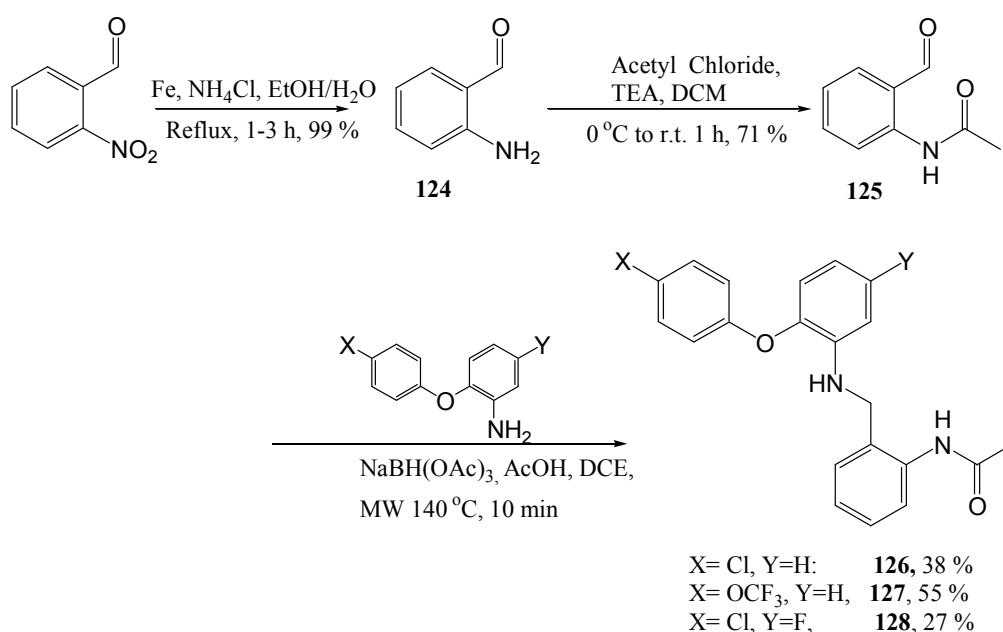
In order to further explore this region of the molecule, the next step was the introduction of a more flexible central region of the molecule, where the restrictive 7-membered piperidine ring has been removed. This introduces a benzylamine template, whilst keeping the overall shape of the molecule similar to that of compounds **122** and **123**. This led to the design of a series of benzylamine linked targets (Figure 8.5).



**Figure 8.5:** Novel benzylamine linked targets.

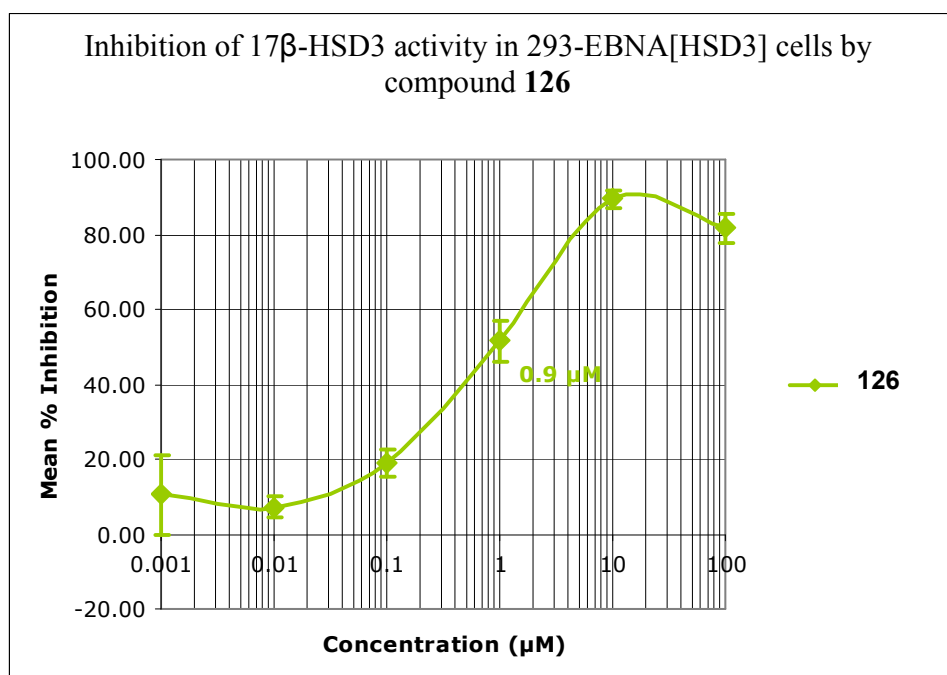
## 8.2 Synthesis of Initial Targets and Biological Data Obtained

The synthesis of the first target was initiated (Scheme 8.1). The commercially available 2-nitrobenzaldehyde was reduced to the corresponding primary amine using iron powder. This intermediate can decompose due to reactions between the amine and the aldehyde functional groups, so must either be stored in the freezer or quickly used in the next step. This step acetylated the nitrogen, which also stabilises the intermediate. The aldehyde can then be reacted with the desired headgroup aniline using the standard reductive amination conditions,<sup>85</sup> to produce the desired final compounds.



**Scheme 8.1:** Synthesis of benzylamine linked targets **126**, **127** and **128**.

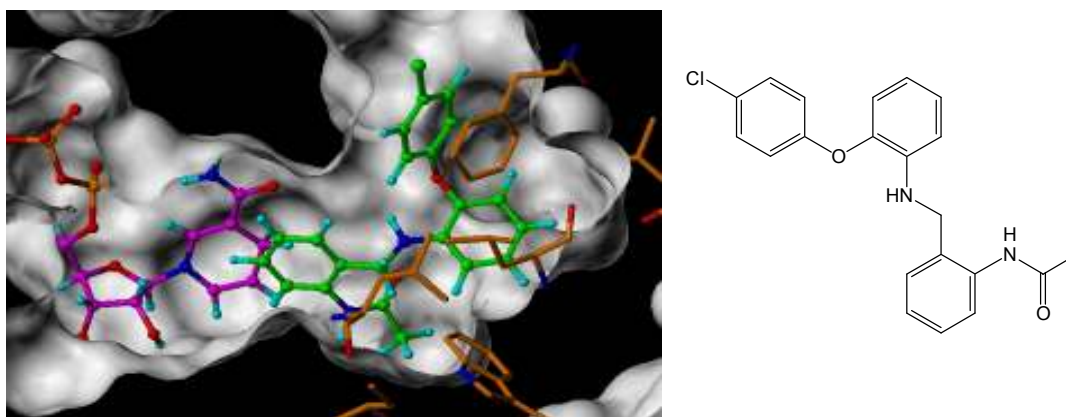
These were the first targets synthesised in this novel series and so, ideally the inhibitory activity of these compounds needed to be established before further compounds were synthesised. Unfortunately, only compound **126** has been subjected to biological testing so far. It showed good inhibitory activity, with an IC<sub>50</sub> value of 0.9 μM (Figure 8.6), which is very similar to the lead compound (**101**, IC<sub>50</sub> = 0.7 μM), thus showing compound **126** to be a very promising lead compound and an excellent starting point for further medicinal chemistry investigations.



**Figure 8.6:** Inhibition of 17β-HSD Type 3 activity by compound **126**, ( $IC_{50} = 0.9 \mu M$ )

### 8.3 *In-Silico* Analysis of the Initial Targets

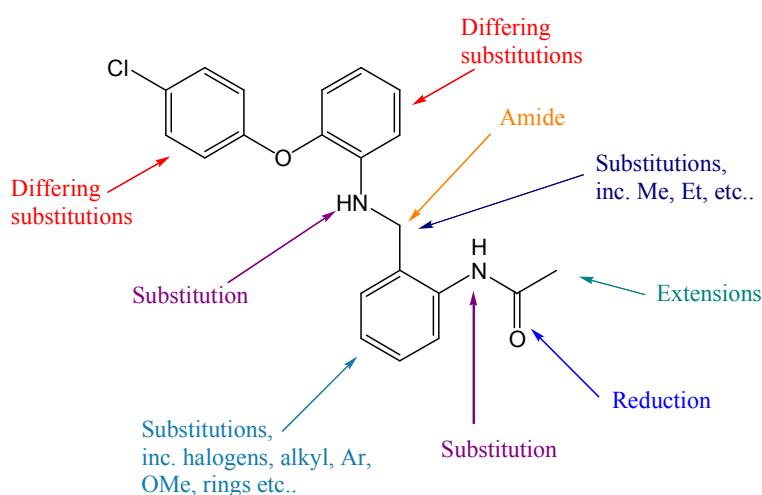
Figure 8.7 shows compound **126** docked into the 17β-HSD Type 3 homology model. It can be seen that the compound is aligned within the active site very differently from the amide and amine linked compounds discussed in Chapters 6 and 7 respectively. The diphenylether hydrophobic headgroup can be seen to lie further within the hydrophobic pocket of the active site, thus, the alignment of the molecule is such that the central aromatic ring lies towards the cofactor at the base of the active site. In the other previously discussed compounds (Chapters 6 and 7), it has been observed that the carbonyl in the acetyl group is usually orientated towards the cofactor. However, in this case the acetyl group is aligned differently. It can be seen that this molecule appears to fit the active site well, but that there is a significant amount of space surrounding the molecule that can be investigated and exploited to improve the activity of the compounds. Although the docking is only a guideline, it is useful to generate hypotheses in early lead optimisation.



**Figure 8.7:** Compound **126** docked in the 17 $\beta$ -HSD Type 3 Homology Model

#### 8.4 Modifications to the Benzylamine Template

The lead compound in this series, compound **126**, showed good inhibitory activity ( $IC_{50} = 0.9 \mu M$ ), and was the starting point for a novel series of compounds. The regions of **126** that required explanation are shown in Figure 8.8. Many of these areas of exploitation were regions of space that could be observed around the molecule in the docking study shown in Figure 8.7. Full investigations into these modifications will allow a very detailed SAR to be acquired. It should also be possible for a QSAR study to be carried out based upon the results from these compounds.



**Figure 8.8:** Compound **126** and the regions modified to prepare a wide range of analogues.

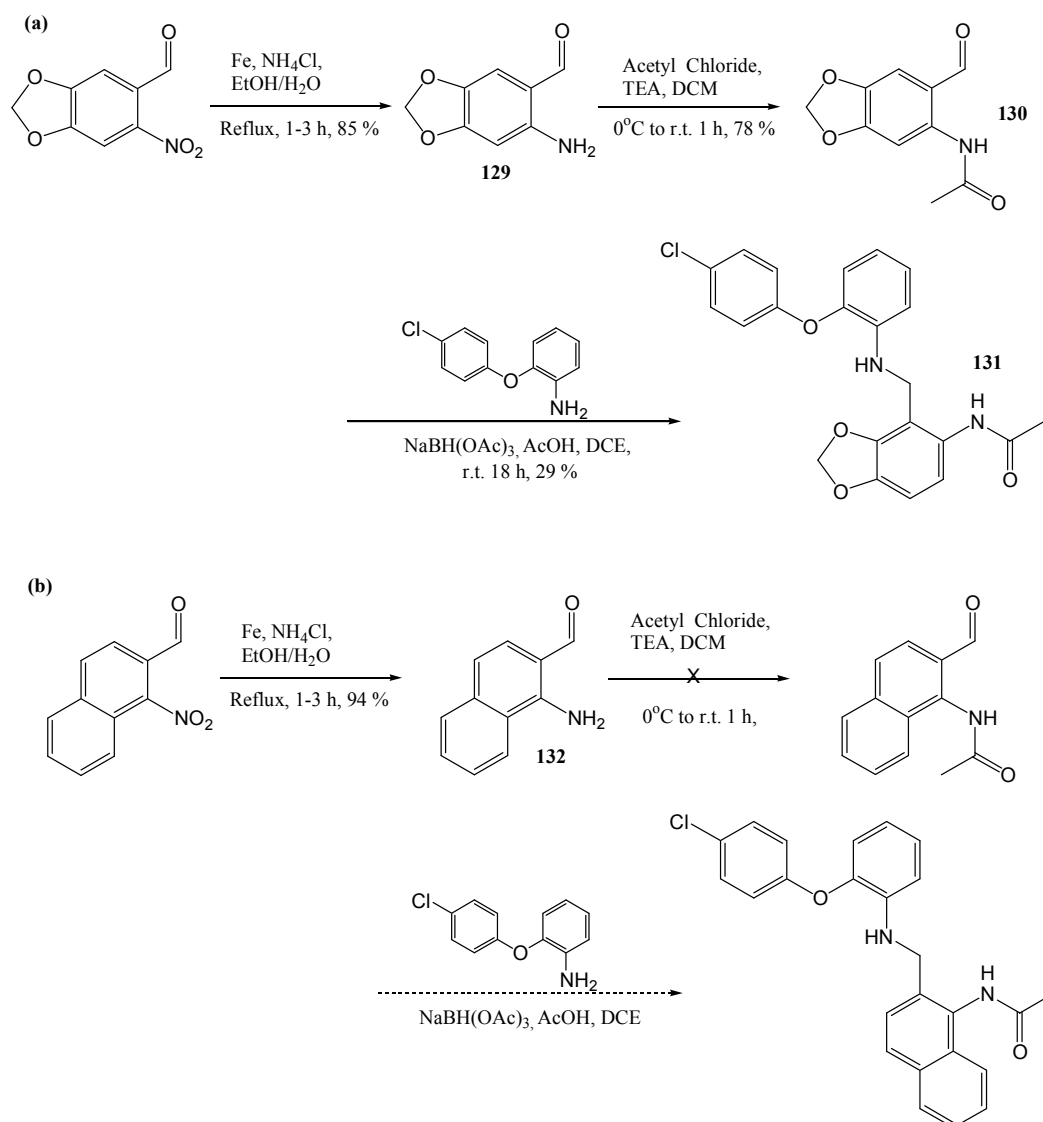
Substitution around the hydrophobic headgroup has already been explored with the preparation of compounds **127** and **128** (Scheme 8.1). The results of the biological

evaluation of these compounds are eagerly awaited. The rest of this Chapter will look at the design and synthesis of other analogues of compound **126**.

#### **8.4.1 Modifications to the Benzylamine Template: Substitutions on the Central Aromatic Ring**

The docking of **126** in the 17 $\beta$ -HSD Type 3 homology model is shown in Figure 8.8. In this figure it can be seen that there is a large amount of space surrounding the central aromatic ring. This region also lies towards the cofactor, and therefore there may be scope for potential interactions in this region. At this stage it was unknown what type of substitutions would be beneficial to activity so the synthesis of a wide range of substitutions was planned, including halogens, heterocycles, naphthyl, alkyl and alkoxy groups.

Initially, the synthesis of these compounds was attempted using the same synthetic route as the synthesis of **126**, (Scheme 8.2a and b). In the case of the methylenedioxy substitution, this synthetic route was successful and compound **131** was synthesised effectively (Scheme 8.2a). It should be noted that the final step in the route, the reductive amination, was now carried out at room temperature as the use of the MW led to the decomposition of starting materials and/or products. However, when the same route was applied to the naphthyl analogue (Scheme 8.2b), it was unsuccessful, as the intermediate **132** was unstable. Even when used immediately or stored in the freezer, it decomposed before acetylation could occur. For this reason a new route was required.

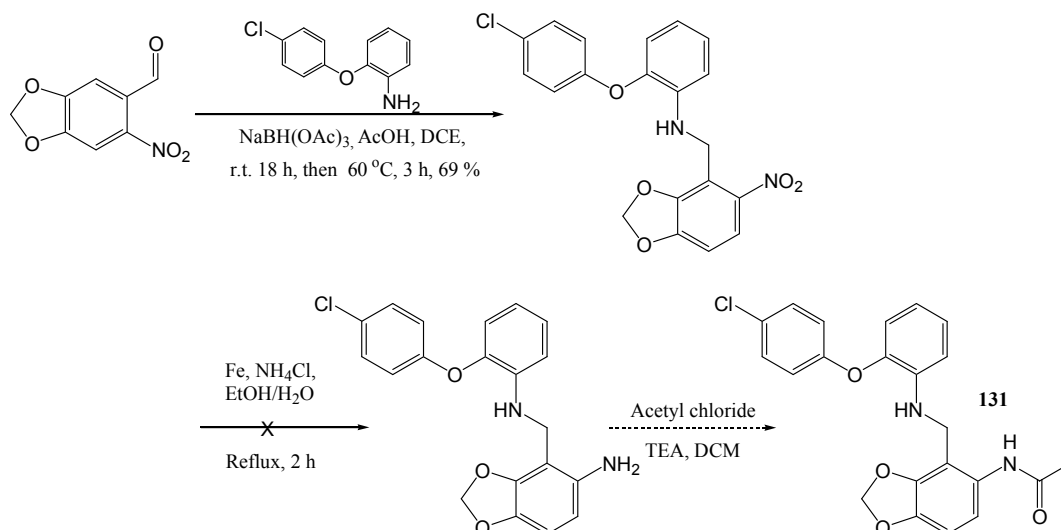


**Scheme 8.2:** (a) Synthesis of **131**, (b) Attempted synthesis of the naphthyl analogue

Investigations were initiated to find a synthetic route that was suitable for a wide range of substituents. The availability of starting materials meant that the methylenedioxy substituent was used in these investigations. In the new route (Scheme 8.3) reductive amination would be the first step and the nitro group would then be reduced and acetylated, thus avoiding the previously problematic intermediate. However, the reduction of the nitro group was unsuccessful and the desired product was not isolated. The iron reduction conditions caused the amine bond in the molecule to break, and only the diphenylether hydrophobic headgroup aniline was isolated. This occurs as the intermediate contains a benzyl protected amine type moiety, thus indicating that the amine bond might be highly sensitive to benzyl deprotection conditions, such as hydrogenation, or in this case iron

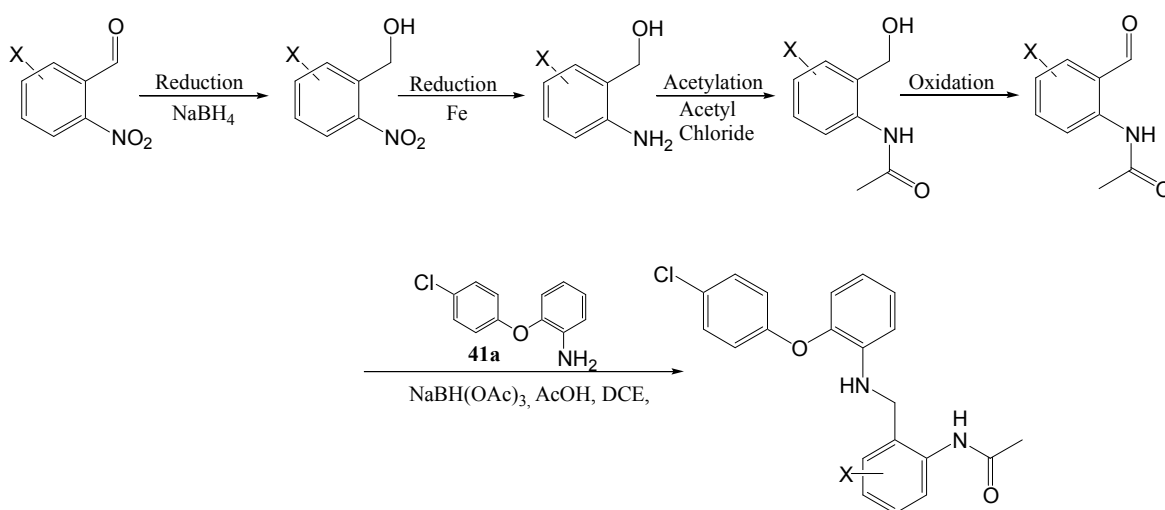


reduction. It is possible that this step could be controlled in order to isolate the desired product, but at this stage a more robust, universal route was required.



**Scheme 8.3:** Second attempted synthetic route to analogues with a substituted central aromatic ring

Another route was initiated. The new route proceeds via the preparation (or purchase) of the nitro benzylalcohol. The presence of the alcohol means that when the primary amine functional group is present the compound is more stable to decomposition than the corresponding aldehyde. Therefore, this was a much more successful synthetic route (Scheme 8.4).

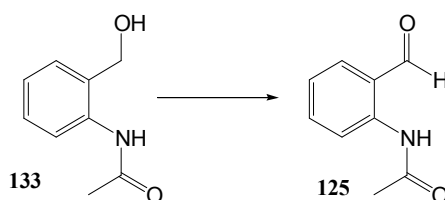


**Scheme 8.4:** New synthetic route to compounds with a substituted central aromatic ring.

Before this route was used to prepare a range of compounds with varying substitution patterns around the central aromatic ring, a certain amount of optimisation was required. The reduction of the aldehyde to the alcohol was carried out using NaBH<sub>4</sub>, with quantitative yields achieved in 2 h. The reduction of the nitro group and the subsequent acetylation were also carried out successfully using the standard iron reduction conditions.<sup>65</sup> The oxidation of the alcohol to the aldehyde required more detailed investigations. There are many available reagents for this oxidation, and some of these were tested to find the most suitable for this application (Table 8.1).

A literature study revealed a new method by Luca *et al*<sup>90</sup> in which trichloroisocyanuric acid (Scheme 8.5) was used to activate TEMPO for the mild and chemoselective oxidation of alcohols (Table 8.1, Entry 1). However, for this particular application it is not a suitable reagent as it also caused a substitution reaction with a chlorine atom, which led to the formation of an alternative product (Scheme 8.5). Although, 5-chloro substitution is one of the targets in this series, this is not a desired method as only the one analogue can be prepared in this way. This is a very interesting reaction and has a possible use as a chlorination procedure, which could potentially be used to introduce chloro substitutions into intermediates, as an alternative to using chlorinated starting materials.

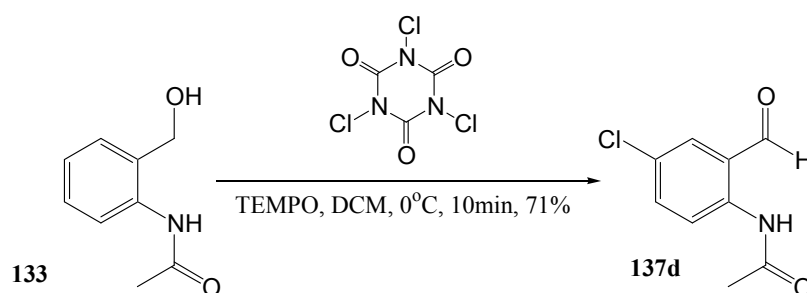
It was hoped that the trichloroisocyanuric acid could be removed from this reaction, and TEMPO could act alone. However, after 3 days, only the starting alcohol was isolated, (Table 8.1, Entry 2).



Entry	Reagent/Conditions	Yield (%) <sup>a</sup>
1	Trichloroisocyanuric Acid, TEMPO, 0°C to r.t. 10 min <sup>90</sup>	<sup>c</sup>
2	TEMPO, DCM, 3 days	<sup>b</sup>
3	SWERN, (oxallyl chloride, DMSO, -50°C, 2 h) <sup>91</sup>	53
4	Mp-Tso-TEMPO, <sup>87</sup> acetonitrile, 4h	66
5	Dess Martin Periodinane (DMP), <sup>92, 93</sup> DCM, 10 min	67
6	MnO <sub>2</sub> , DCM, 18 h	71

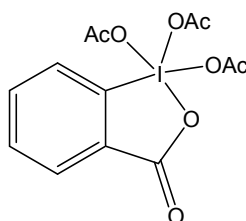
<sup>a</sup> Isolated Yield. <sup>b</sup> Only starting material isolated. <sup>c</sup> Incorrect product isolated, mono-chloro substitution occurred.

**Table 8.1:** Oxidation conditions investigated.



**Scheme 8.5:** Formation of alternative chloro substituted product.

Four successful methods for this oxidation were discovered (Entries 3-6), all producing the correct product in similar yields. The least successful method was the SWERN oxidation,<sup>91</sup> as only a 53 % yield was obtained. As other, more facile options are available, the SWERN reaction is not the method of choice. All the other three successful methods, resin bound MP-TsO-TEMPO,<sup>87</sup> Dess Martin Periodinane (DMP)<sup>92, 93</sup> and  $\text{MnO}_2$ , would be a suitable choice for this reaction. DMP (Figure 8.9) was chosen as the method of choice,<sup>92, 93</sup> because in these initial investigations it gave the most reproducible results, did not require anhydrous conditions and the reagent is easy to remove at the end of the reaction, through a simple aqueous work up and standard flash chromatography.

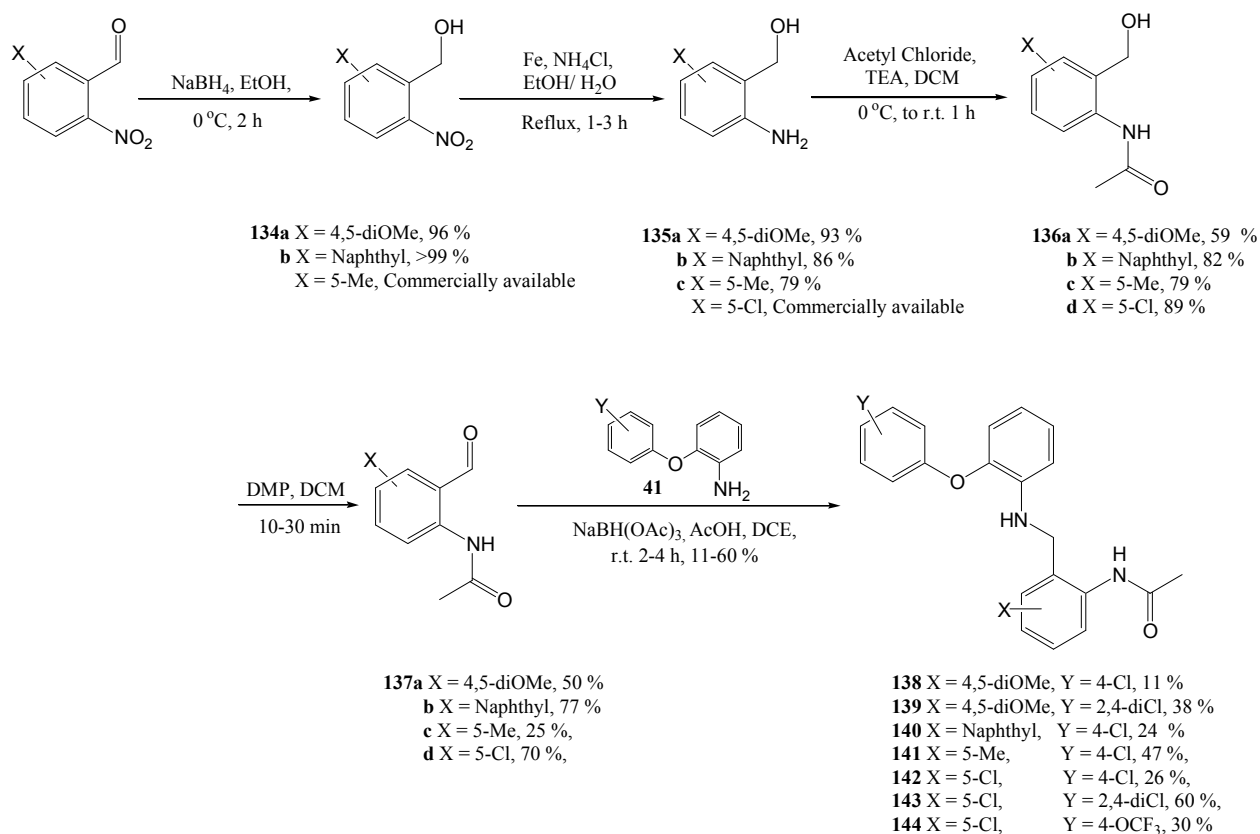


**Figure 8.9:** Dess-Martin Periodinane

DMP was used to oxidise the alcohol in the synthesis of all targets in this series, (Scheme 8.6). Unfortunately, the reliable results observed in the test reactions were not conserved, and the yields varied from 25-77 %. If it is required to repeat the synthesis of any of these compounds or more compounds in this series are required, it is recommended that  $\text{MnO}_2$  is investigated further as a more reproducible reagent for the oxidation. This may produce better results or the results of the oxidation may be substrate dependent.

There are many different suitable starting materials with differing substitution patterns that are commercially available, either as the 2-nitrobenzaldehyde, the 2-nitrobenzylalcohol or the 2-aminobenzylalcohol. The availability of the commercial starting materials dictated at which step in the route synthesis began. For example, the 4,5-dimethoxy substitution

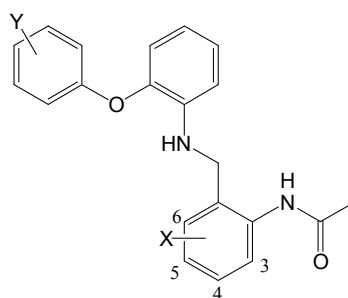
pattern was only available as the 2-nitroaldehyde, so the full 5 step synthesis was required. In contrast, the 5-chloro substitution was available as the 2-aminobenzylalcohol, so only the final 3 steps were required.



**Scheme 8.6:** Synthetic route for the synthesis of analogues with various substitutions around the central aromatic ring

This synthetic route has led to a wide range of **126** analogues with various substitutions around the central aromatic ring. Eight novel compounds were prepared in this series. These are listed in Table 8.2. They have all been submitted for biological evaluation. However, only some results have so far been obtained. These are shown in Table 8.2.

It can be seen from the few available biological results that substitutions around the central aromatic ring are well tolerated, as even the dioxy substitution on **131** does not have a substantially detrimental affect upon the activity. Although the functionalities are tolerated, they do not seem to lead to the desired increase in activity, as **126** is still the most active compound in this series so far. This may, of course, change once full biological data is obtained for all compounds in this series.

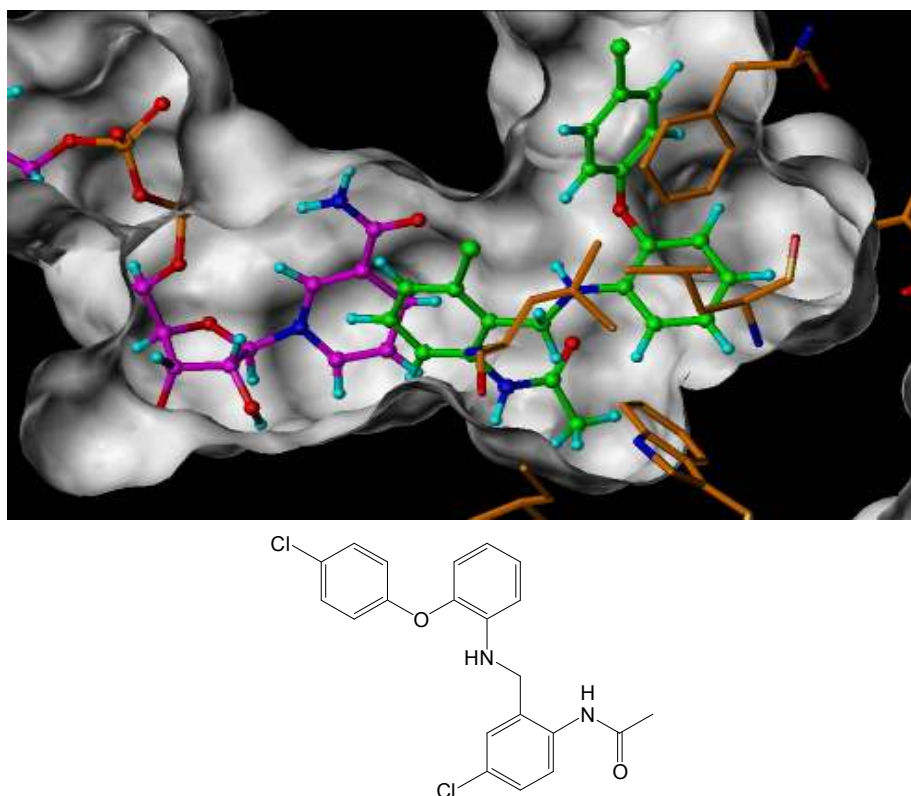


Compound No.	X	Y	% Inhibition (at 10 $\mu$ M). <sup>a</sup>
138	4,5-diOMe	4-Cl	<sup>b</sup>
139	4,5-diOMe	2,4-diCl	<sup>b</sup>
140	naphthyl	4-Cl	<sup>b</sup>
141	5-Me	4-Cl	<sup>b</sup>
142	5-Cl	4-Cl	97 (IC <sub>50</sub> = 1.9 $\mu$ M)
143	5-Cl	2,4-diCl	<sup>b</sup>
144	5-Cl	4-OCF <sub>3</sub>	<sup>b</sup>
131	Dioxyl <sup>c</sup>	4-Cl	86
126	H <sup>d</sup>	4-Cl	87 (IC <sub>50</sub> = 0.9 $\mu$ M)

<sup>a</sup> Results obtained from the TLC assay. Mean of at least 2 measurements with typically a SD of  $\pm 5$  %. <sup>b</sup> Not determined. <sup>c</sup> Synthesised using a different route, see Scheme 8.2a. <sup>d</sup> Synthesised using a different route, see Scheme 8.1.

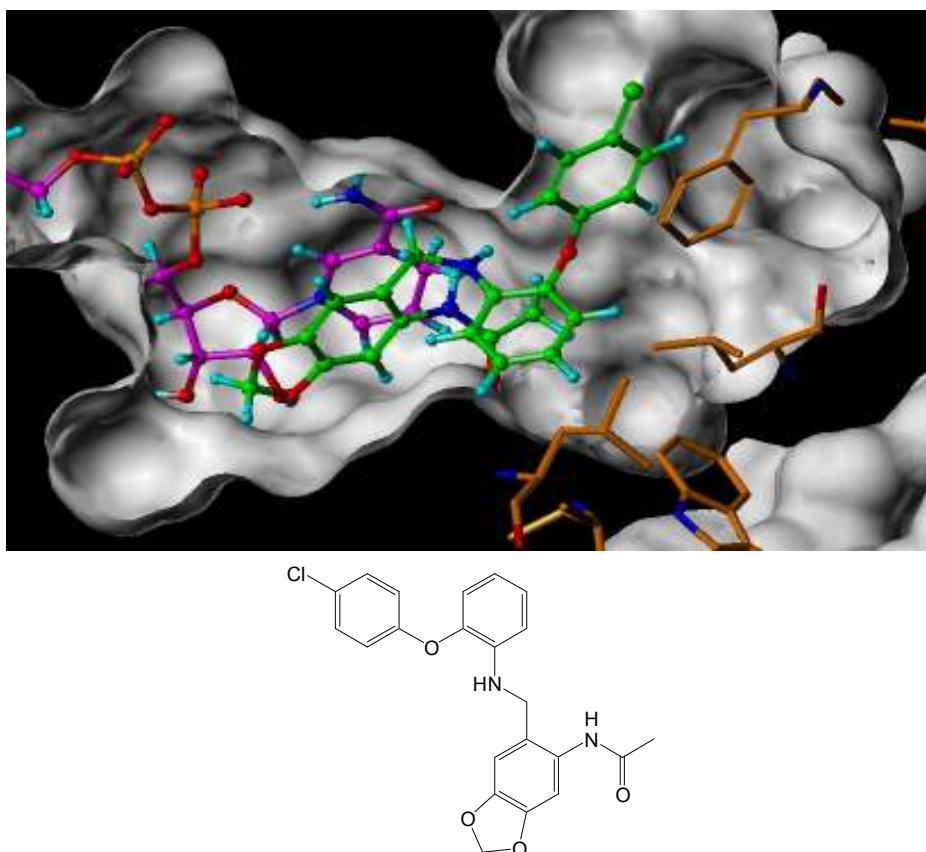
**Table 8.2:** Full compound list and biological data obtained for **126** analogues with substitutions around the central aromatic ring.

Some interesting observations can be made from the docking studies of these compounds, (Figures 8.10 and 8.11). Some substitutions, such as the chloro substitution in compound **142** (Figure 8.10) do not seem to affect the overall position of the molecule within the active site, when compared to **126**. This might explain why little difference in inhibitory activity is observed.



**Figure 8.10:** Compound **142** docked into the 17 $\beta$ -HSD Type 3 homology model.

However, a considerable change in alignment is observed in the docking study of the dioxyl substituted compound **131** (Figure 8.10), as the compound no longer is aligned in the same way as **126** (Figure 8.7). It appears that the dioxyl is interacting strongly with the cofactor within the active site. This interaction is strong enough to realign the molecule, as the hydrophobic headgroup is no longer in a hydrophobic pocket of the enzyme. It could be assumed that this change in alignment would greatly alter the biological activity. However, the result of compound **131** is not substantially different to **126** and both have a percentage inhibition of approximately 86 %. Unfortunately the IC<sub>50</sub> of compound **131** has not been established so far, so a more definite conclusion cannot be made.



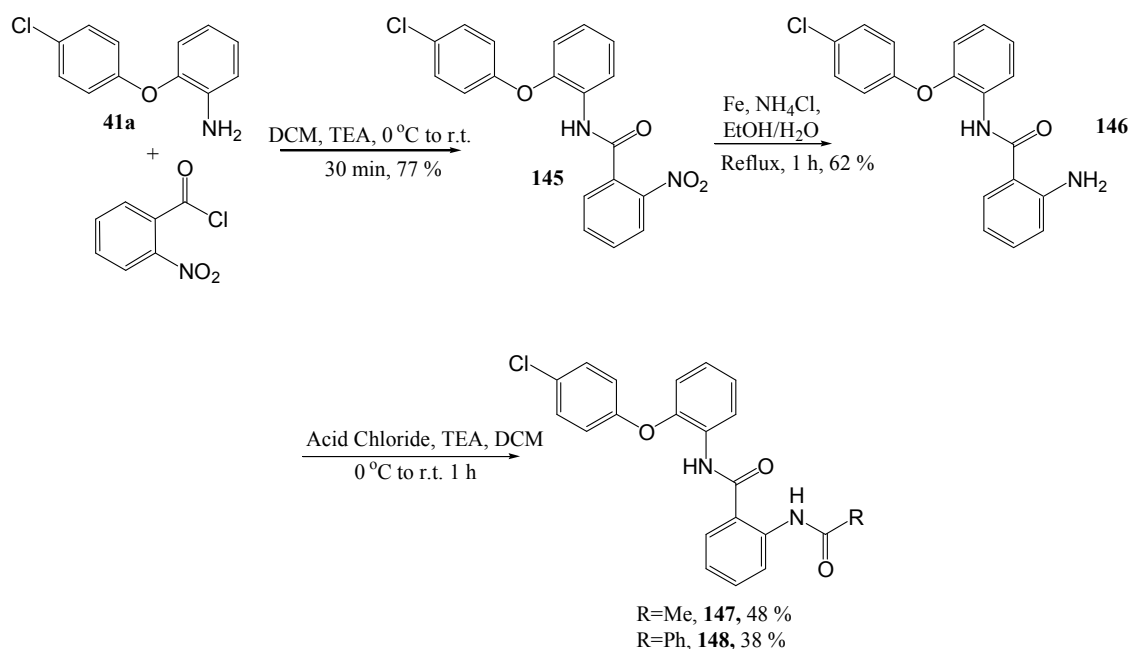
**Figure 8.11:** Compound **131** docked into the 17 $\beta$ -HSD Type 3 homology model.

Once the full biological data have been obtained, a much clearer SAR will be gained for this region. So far it can be established that substitutions around the central aromatic ring are not detrimental to activity, although the substitutions tested so far have not shown beneficial inhibitory effects. This means that this region may require further investigation.

#### 8.4.2 Modifications to the Benzylamine Template: Synthesis of Benzamide Analogues

As shown in Chapter 5, amide linked compounds were one of the initial targets for this project. Therefore, the preparation of amide linked analogues of compound **126** would allow SAR comparisons to be made with the amide linked compounds discussed in Chapter 6. This would investigate whether the aromatic linker is beneficial to activity compared with a piperidine linker. The synthesis of these analogues was initiated (Scheme 8.7). The aniline headgroup (**41a**) was reacted with 2-nitrobenzoylchloride to form the desired amide bond. Reduction of the nitro group afforded the primary amine to react with the desired acid chloride. This primary amine intermediate (**146**) was split into two batches and

reacted with 2 different acid chlorides (acetyl chloride and benzoyl chloride) to form a small series comprising two final compounds, **154** and **155**. These compounds have been submitted for biological testing, but the results have not yet been obtained.



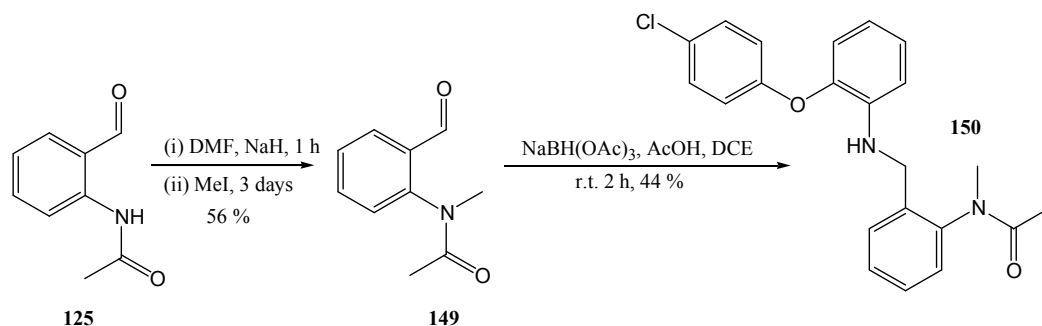
**Scheme 8.7:** Synthesis of benzamide linked compounds **147** and **148**.

#### 8.4.3 Modifications to the Benzylamine Template: Substitutions on the Nitrogen Atoms

In any medicinal chemistry programme it is important to investigate any potential hydrogen bond interactions that may be occurring between the molecule and the active site of the enzyme. Hydrogen bonds most commonly occur between functional groups containing nitrogen or oxygen atoms, which can undergo hydrogen bond donor-acceptor interactions. In this series, the main potential hydrogen bond regions are the nitrogen atoms in the amine and the amine bond and also the oxygen atoms in the diphenylether headgroup and the amide bond. The introduction of a non H-bonding substitution on to either/both nitrogen atoms will help to determine if either NH plays an important part in enzyme-inhibitor interactions as a hydrogen bond donor. The synthesis of a series of *N*-methylated compounds was initiated to remove the hydrogen donating ability of the NH. In order to methylate the amide nitrogen alone, it was thought necessary to carry out the methylation on the *N*-(2-formyl-phenyl)-acetamide intermediate (**125**), before the reductive amination with the headgroup. This methylation was successful and the methylated intermediate was

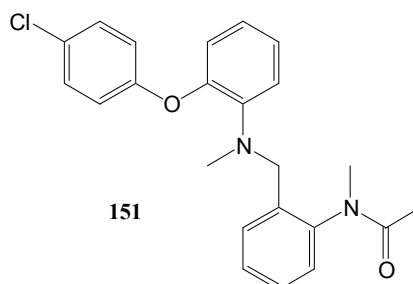


isolated in 56 % yield. This was then reacted with the desired headgroup to produce **150** in 44 % yield (Scheme 8.8).



**Scheme 8.8:** Synthesis of compound **150**.

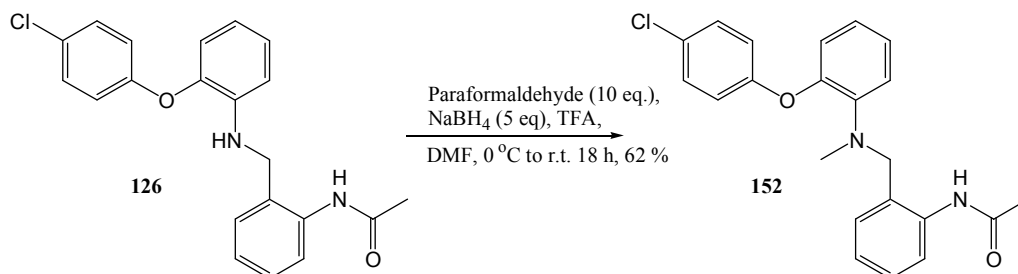
Another method of methylation was investigated as a possible way to synthesise the desired compounds directly from **126**. By using different ratios of sodium hydride and methyl iodide it was hoped that the three desired methylated analogues could be synthesised. However, it was not straightforward, as the desired product was not always obtained as predicted. Methylation of both the amine and the amide nitrogen simultaneously was possible via this route (23 %, **151**, Figure 8.12), even though the major product of the reaction was actually the mono-methylated product previously synthesised, (43 %, **150**, Scheme 8.8). Compound **151** has no hydrogen bond donors available for enzyme-inhibitor interactions, so the biological activity will show whether these are important interactions for inhibitory activity. The attempted synthesis of the mono-methylated amine analogue was carried out. Unfortunately, this did not produce the desired product and only the mono-methylated amide product previously synthesised (**150**) was formed. An alternative route was therefore required for this analogue.



**Figure 8.12:** Di-methylated product, **151**.

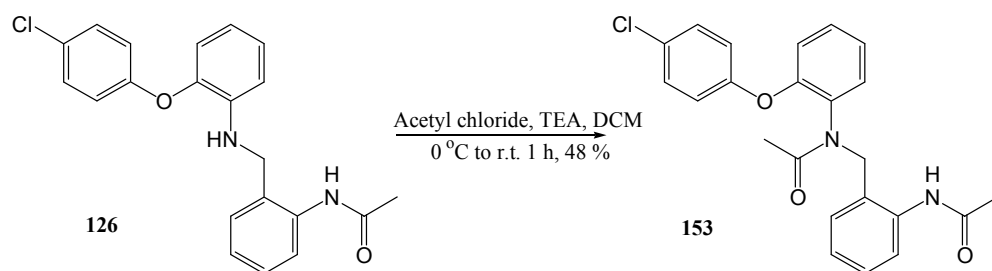
Methylation of the amine nitrogen alone was possible via a different method, directly from compound **126** (Scheme 8.9). The compound was reacted with paraformaldehyde to give the

*N*-formyl product *in-situ* which was then reduced with NaBH<sub>4</sub>/TFA to form the desired product (**152**) in a 62 % yield.<sup>94</sup>



**Scheme 8.9:** Synthesis of compound **152**

The possible effects of the introduction of an acetyl group were also investigated by the synthesis of **153** (Scheme 8.10). It was a one step procedure to the product (**153**), which was obtained in a 48 % yield.



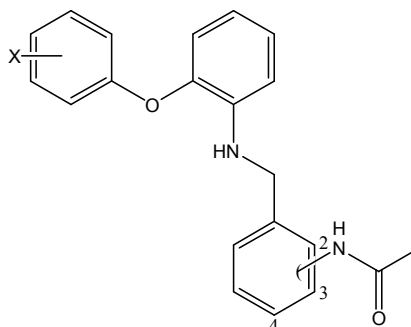
**Scheme 8.10:** Synthesis of compound **153**

There are now 4 compounds in this series, all of which have been submitted for biological testing. However, the results have not yet been obtained. It is hoped that the biological results will allow for a direct comparison with the parent template, **126**, and will give a good indication of the SAR surrounding the nitrogen atoms in the molecule and the potential for hydrogen bond donor interactions.

#### 8.4.4 Modifications to the Benzylamine Template: 2, 3 and 4 *N*-Acetamide Analogues

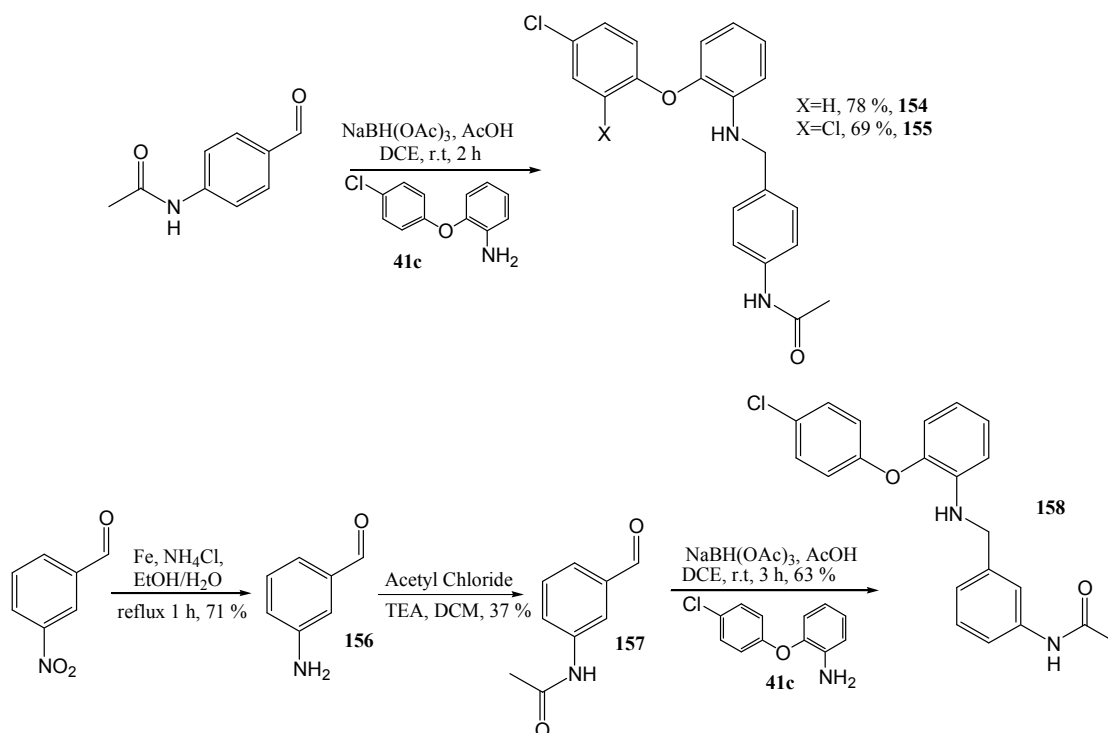
This benzylamine series of compounds aimed to be a more flexible version of **122** and **123** (Figure 8.3) by removing the restriction caused by the piperidine ring. However, the docking of the original target **126** (Figure 8.8) showed that the alignment of the compound was different to that which had been expected. Activity may be improved by redesigning

the compound to allow it to take up an alignment more similar to the original amide or amine linked compounds (Figures 6.2 and 7.2). It was hypothesised that this may be achieved by migrating the *N*-acetamide around the central aromatic ring, from the original 2-position of **126** to the 3 or 4 position (Figure 8.13).



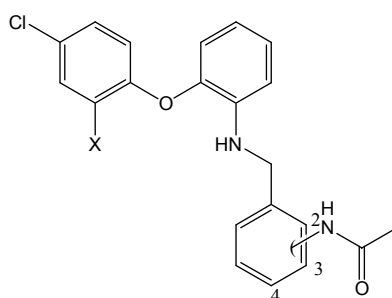
**Figure 8.13:** New targets with a migrated *N*-acetamide

Compound **126** is the 2-substituted compound, so therefore there were two further compounds that required synthesis in this series for an SAR to be established. The synthesis of these two analogues was initiated (Scheme 8.11). For the 4-substituted analogue, the 4-*N*-acetylated aldehyde was commercially available, thus meaning that the final compound, **154**, could be synthesised in one step by reductive amination with the 4-chloro diphenylether aniline and isolated in 78 % yield. The 2,4-dichloro analogue was also synthesised, **155** (isolated yield 69 %). For the 3-substituted analogue, the 3-*N*-acetylated aldehyde required synthesis from the commercially available 3-nitroaldehyde. In this particular case it was possible to reduce the nitro group with the aldehyde present and then acetylate the primary amine. This is not the ideal synthetic route as it is low yielding due to the decomposition of the 3-aminoaldehyde intermediate. However, the desired product was obtained, which was then reacted with the 4-chloro headgroup aniline to produce compound **158** in a 63 % yield.



**Scheme 8.11:** Synthesis of **154**, **155** and **158**.

These 3 compounds have been submitted for biological testing, and the available results are shown in Table 8.3. The results show that, contrary to the hypothesis, the migration of the *N*-acetyl group does not lead to increased inhibitory activity and in this series the 2-acetyl substitution remains the most active inhibitor.



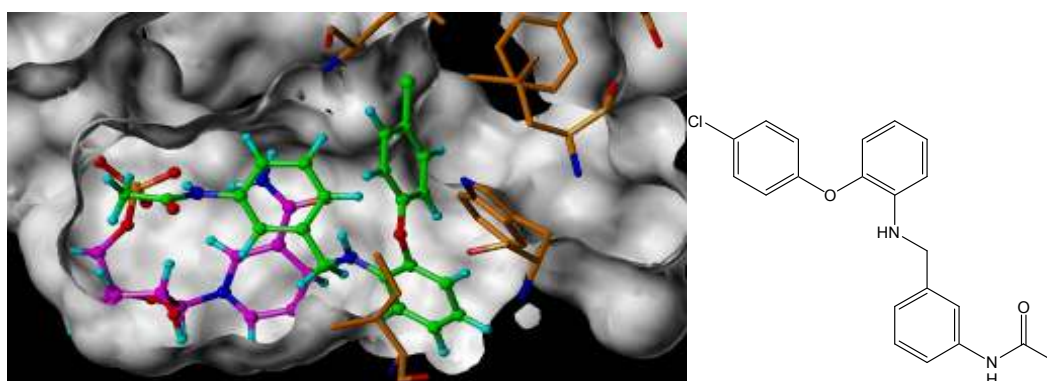
Compound no	2/3/4	X	% Inhibition (at 10 $\mu$ M). <sup>a</sup>
126	2	H	87
158	3	H	53
154	4	H	29
155	4	Cl	<sup>b</sup>

<sup>a</sup> Results obtained from the TLC assay. Mean of at least 2 measurements with typically a SD of  $\pm 5$  %. <sup>b</sup> Not determined.

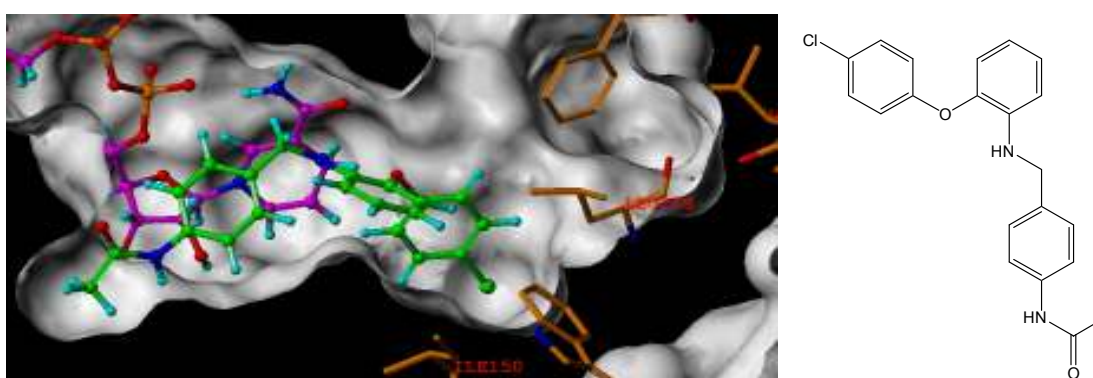
**Table 8.3:** The available biological data for compounds with a migrated *N*-acetamide group.

These compounds have been analysed *in-silico* and the docking studies with the 17 $\beta$ -HSD Type 3 homology model are shown in Figures 8.15 and 8.16. It can be seen that the migration of the *N*-acetamido group to both the 3- and 4- positions causes the compounds to be pulled towards the cofactor, thus meaning that the hydrophobic headgroup is no longer orientated in the hydrophobic pocket of the enzyme. In the case of the 3-substituted compound, (**158**, Figure 8.14), the *N*-acetamide is drawn towards the cofactor, and the hydrophobic head group is aligned into a different pocket of the enzyme. In the 4-substituted compound (**154**, Figure 8.15), the same phenomenon seems to have occurred, except that this has also changed the alignment of the headgroup. These changes in alignment may explain the reasons for the lower activity observed with these compounds compared to the 2-substituted compounds.

The lack of activity suggests that this series does not command further investigation. It should be noted that a change in another part of the template may improve activity for these compounds so, at a later date, this series may be worth exploring again.



**Figure 8.14:** Compound **158** docked into the 17 $\beta$ -HSD Type 3 homology model.



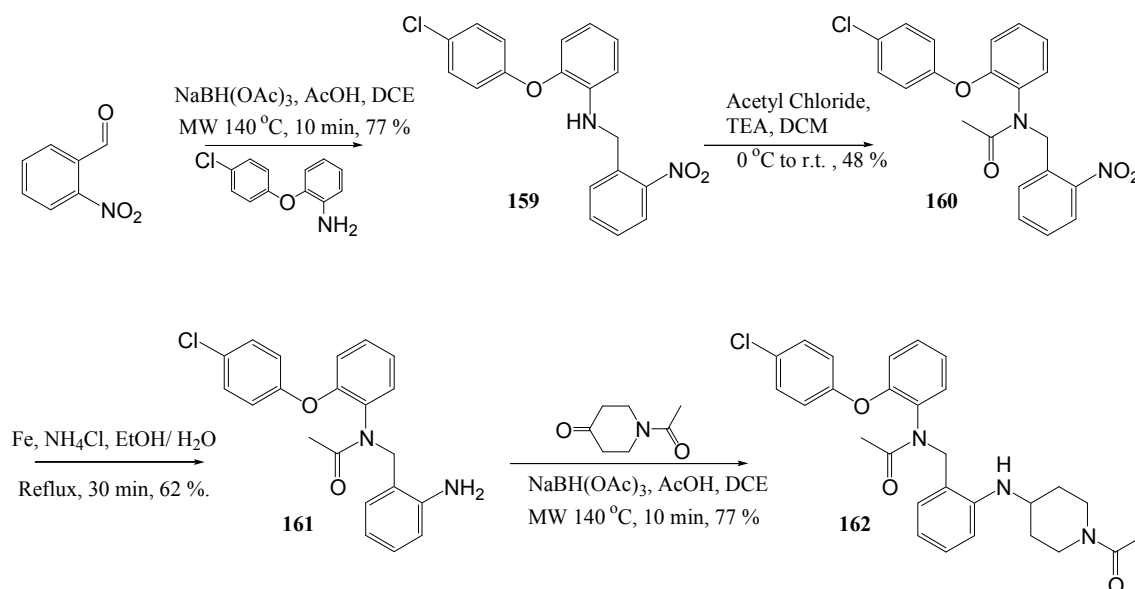
**Figure 8.15:** Compound **154** docked into the 17 $\beta$ -HSD Type 3 homology model.

#### 8.4.5 Modifications to the Benzylamine Template: Extended Analogues

Many different regions of this template have been explored and so far, with the available biological results no improvement in activity over the original compound **126** have been observed. In previous series, (see Chapters 6 and 7), it was hypothesised that increasing the length of the potential inhibitors might help to fill the space in the active site and allow the inhibitors to interact with both the hydrophobic head group and the cofactor simultaneously. In the case of this benzylamine series, it is not as clear from the docking studies whether this extension will help, as the compound is aligned differently. It is possible that the extension of this series will cause the molecule to be realigned within the active site, and this may increase the activity. For these reasons, the synthesis of extended benzylamine linked analogues was initiated (Scheme 8.12).

The synthetic route was different to those already used within this series of compounds. Since the point of diversification is desired to be the final step in the synthesis common intermediates are used for the majority of the synthetic route. Hence, the first step was the

reductive amination between the headgroup aniline and 2-nitrobenzaldehyde. This was carried out using the standard MW conditions,<sup>85</sup> and the product was isolated in an excellent 77 % yield. At this point, it was thought that the aniline nitrogen required some kind of protection to ensure that it did not interfere with any reactions at the primary amine at a later stage in the synthetic route. The first choice for this protection was an *N*-Boc protecting group. However, only starting material was isolated from the reaction with di-*t*-butyl-dicarbonate so another method was required. The use of a small acetyl group was chosen, meaning that the formation of the protected amine was successful (45 % yield). Deprotection would, however, be more problematic, although compounds with an acetyl group present are still potential inhibitors. The reductive amination with 1-acetyl-4-piperidone produced the desired compound, **162** in an excellent yield (77 %).

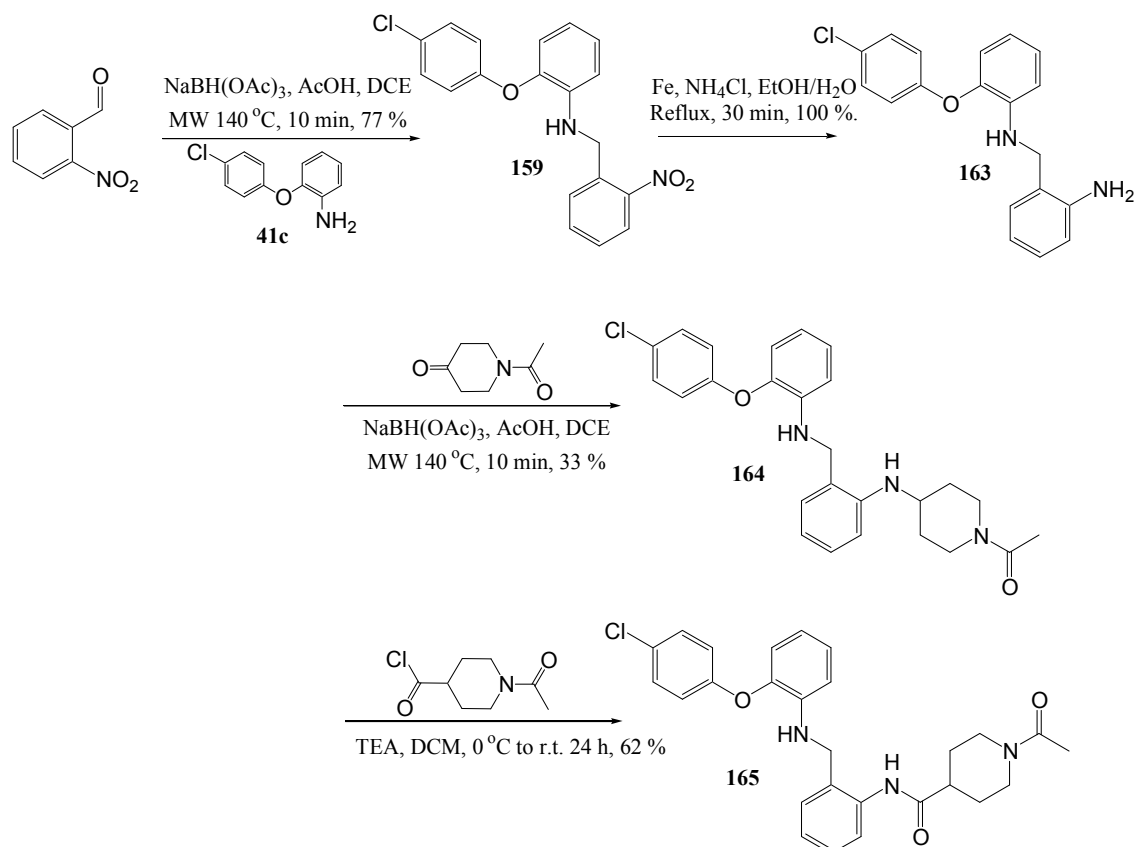


**Scheme 8.12:** Synthetic route to the extended analogue **162**.

It was then decided to investigate whether this *N*-acetyl protection was required, or whether the steric constraints and decreased reactivity of the aniline NH were enough to stop these potential side reactions occurring. Therefore, the synthetic route was initiated again (Scheme 8.13). It turned out that when the reductive amination was carried out with 1-acetyl-4-piperidone the reaction occurred successfully, albeit with a low yield, to produce the desired compound, **164**. There was no sign that the aniline nitrogen had been involved in the reaction.

Previously extended compounds in the original amine and amide linked series had been extended using an amide bond and therefore this would be an interesting modification to

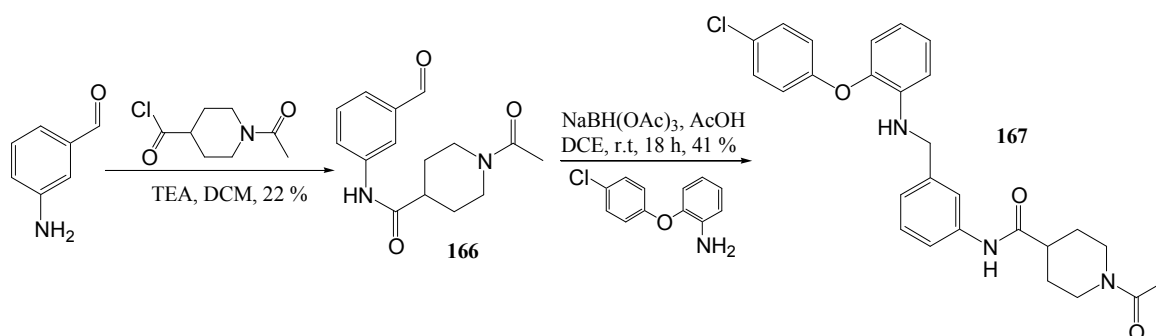
include in this series. The same primary amine intermediate (**163**) was therefore reacted with 1-acetyl-piperidine-4-carbonyl chloride to produce compound **165**, a compound extended via an amide bond (Scheme 8.13).



**Scheme 8.13:** Synthesis of compounds **164** and **165**.

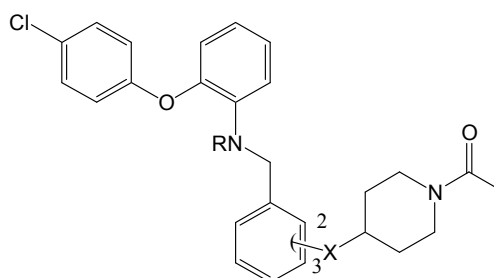
It was also decided to synthesise an extended compound which has had the *N*-acetamide group migrated around the ring to the 3-position (Scheme 8.14). This final compound could be synthesised in a manner similar to the normal length analogues in the extended analogue series, except at the acetylation step, 1-acetyl-piperidine-4-carbonyl chloride was used as the acid chloride. This synthetic route gave the desired product **167**.





**Scheme 8.14:** Synthesis of compound **167**.

The extended analogues were submitted for biological testing and the whole series has been tested for inhibitory biological activity. The results are shown in Table 8.4, and show some interesting patterns. The extension of this series via an amine bond (**162** and **164**) is detrimental to activity, reducing the percentage inhibition to 55 and 36 % respectively. Extension of this series via an amide bond (**165** and **167**) was much more promising, with percentage inhibitions of 91 and 97 % respectively. The  $IC_{50}$  values for these two compounds were also established, and are shown in Table 8.4. They both show a reduction in activity compared to compound **126** ( $IC_{50}$  = 0.9  $\mu$ M), but in the case of compound **167** it is only a small reduction. The activity is highly comparable to that of **126**.

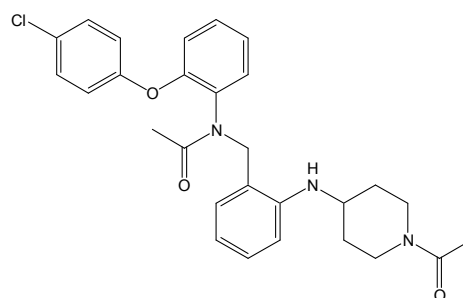
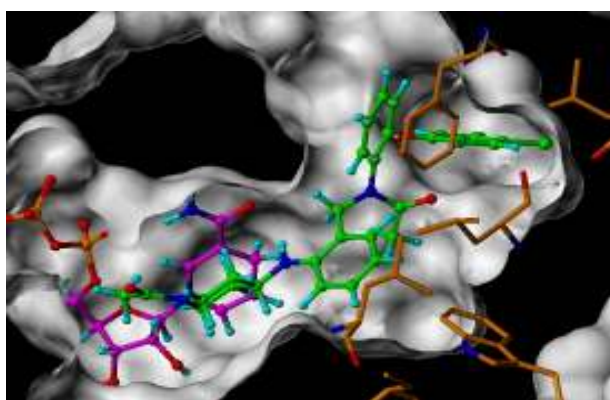


Compound no.	R	X	2/3	% Inhibition (at 10 $\mu$ M). <sup>a</sup>	$IC_{50}$ ( $\mu$ M)
<b>162</b>	Ac	NH	2	55	<sup>b</sup>
<b>164</b>	H	NH	2	36	<sup>b</sup>
<b>165</b>	H	NHCO	2	91	3.9
<b>167</b>	H	NHCO	3	97	1.1

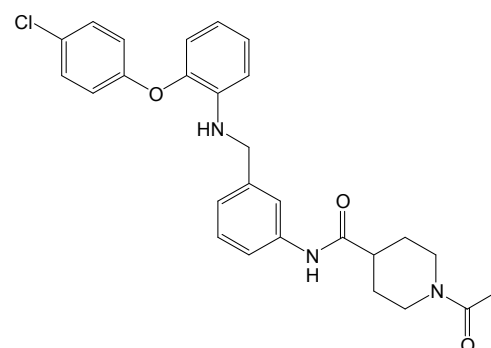
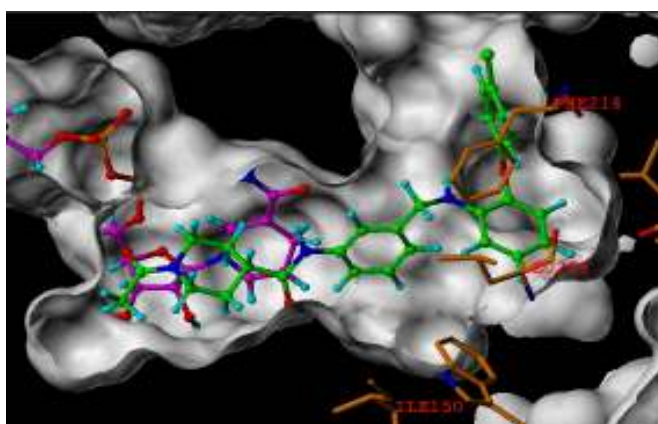
<sup>a</sup> Results obtained from the TLC assay. Mean of at least 2 measurements with typically a SD of  $\pm 5$  %. <sup>b</sup> Not determined.

**Table 8.4:** Biological results obtained for the extended analogues.

The extended compounds were analysed *in-silico* and the docking of compounds **162** and **167** is shown in Figures 8.16 and 8.17. These show that both these compounds are aligned very differently to the shorter compounds, such as compound **126** (Figure 8.7). In both cases, the extra length of the compounds means that the hydrophobic head groups lies within the hydrophobic pocket. From these docking figures alone, it is hard to see why there is such a difference in activity between the two compounds in this series. Both have a similar alignment and are similar in length. It can be hypothesised that the difference is due to a favourable interaction between the carbonyl of the central amide in **167**, whereas this is absent in **162**. This slight increase in length may also mean that the carbonyl of the *N*-acetamide group can form a better interaction with the cofactor at the base of the active site. The 3-substituted compound **167** is more linear than the corresponding 2-substituted compound **165**. This difference in linearity may explain the difference in activity between them. The active site has a narrower region in the centre. Therefore, the more linear compounds may fit better within this channel unaffected by unfavourable steric constraints.



**Figure 8.16:** Compound **162** docked into the 17 $\beta$ -HSD Type 3 homology model.



**Figure 8.17:** Compound **167** docked into the 17 $\beta$ -HSD Type 3 homology model.

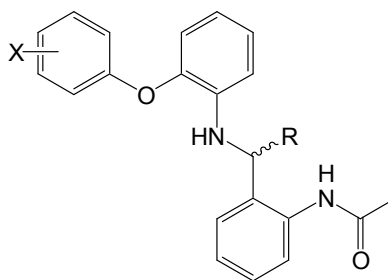
Extension of the compounds may also have the added advantage that if the compounds are a more restricted fit within the 17 $\beta$ -HSD Type 3 active site, they may be more selective over other 17 $\beta$ -HSD isozymes. However, there is no evidence that this is relevant to this series of compounds.

Once this benzylamine template has been fully explored, the biological results will enable a satisfactory SAR to be developed. The extension of the compounds via an amide bond to a piperidine ring is a feature of the series that should be retained, and if applicable, combined with other favourable features to see if there are additive effects on potency. The combination of successful features from the SAR exploration should lead to more active compounds will be synthesised in the future.

#### **8.4.6 Modifications to the Benzylamine Template: Substitution on to the CH<sub>2</sub> of the Benzylamine Bond.**

##### **8.4.6.1 Introduction**

As discussed at the beginning of this Chapter, a favourable feature of the Schering-Plough Competitor compounds is the (*S*)- lipophilic *t*-butyl group on the piperidine ring. It has been demonstrated that this region leads to a very favourable increase in inhibitory activity.<sup>46</sup> It is therefore likely to be beneficial to incorporate a hydrophobic group that can mimic this region into the design of novel inhibitors. Within this series of benzylamine targets there are a few regions which may be able to mimic this group, including the central aromatic ring itself and also substitutions around the central aromatic ring. This region has been previously investigated and biological data are eagerly awaited. It is also possible that the inclusion of alkyl groups on to the CH<sub>2</sub> of the amine bond may have favourable hydrophobic interactions. Therefore, a novel range of targets with substitutions in this region was designed (Figure 8.18).

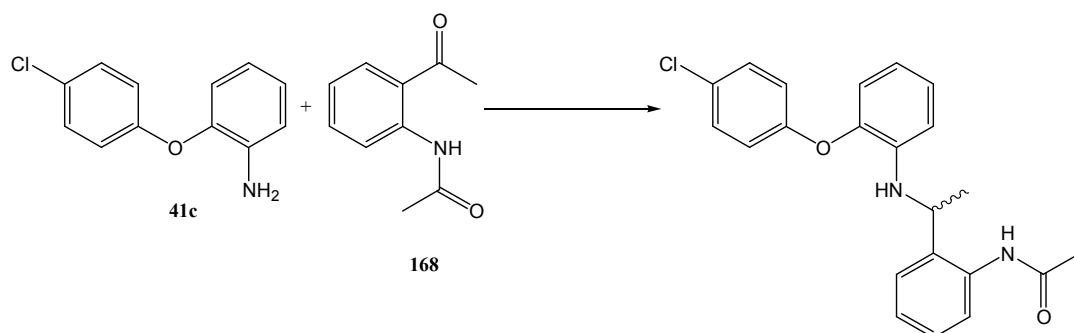


Where R= Me, Et, Pr, Ph, allyl, vinyl, amongst others.

**Figure 8.18:** Novel range of substituted targets.

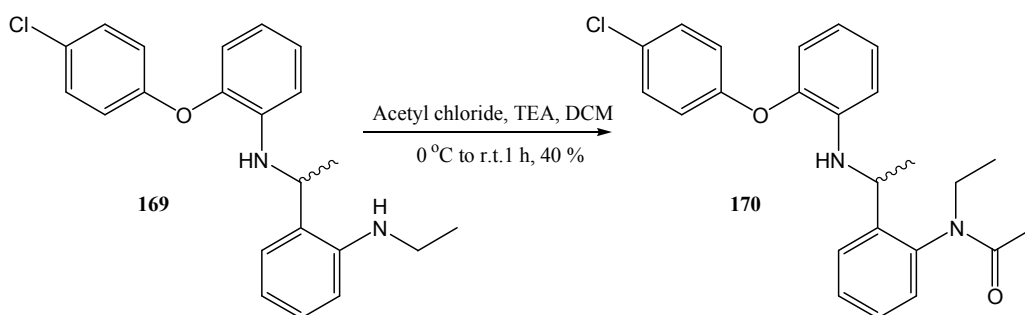
#### 8.4.6.2 Route A: Attempted Reductive Amination with Ketone.

The introduction of a methyl substituent was the initial target. The synthesis was started from the commercially available starting material, 2-aminoacetophenone. This was acetylated and then subjected to a reductive amination with the diphenylether aniline. However, this reaction did not proceed as expected because the standard reductive amination conditions discussed in Chapter 7 were unsuccessful (Table 8.5),<sup>85</sup> even when an extended reaction time was used (Entry 1). The same lack of reactivity was observed when traditional Dean-Stark conditions were used (Entry 2). Many different sets of conditions for an indirect reductive amination were then attempted (Entries 3-6). Using  $\text{TiCl}(\text{O}^i\text{Pr})_3$  as the Lewis acid, it was attempted to form the imine, which could then be reduced *in-situ* to form the desired product. Unfortunately, none of the sets of conditions attempted led to the desired product. In some cases, an interesting phenomenon was observed. The use of sodium borohydride as the reducing agent (Entry 3) led to the formation of an alternative product (**169**, Scheme 8.15), where the two starting materials have undergone a reductive amination as intended. However, the *N*-acetyl amide bond has also been reduced to an ethyl group. Attempts to control this reaction, by using shorter reaction times or cooler reaction conditions were not successful, as only starting materials were isolated. The synthesis of compound **169** may be beneficial as it will help to investigate the effects of the amide bond upon enzyme binding. Compound **167** was then acetylated to produce **170** (Scheme 8.15), where the standard *N*-acetyl group is now present, but the NH of the amide is substituted with an ethyl group. This will give further SAR around this nitrogen atom, as previously discussed.



Entry	Conditions	Result
1	Standard MW conditions <sup>85</sup> , 140 °C, 30 min	SM only
2	<i>p</i> -TSA, 4Å MS, Dean-Stark reflux 18 h	SM only
3	TiCl(O <sup>i</sup> Pr) <sub>3</sub> , r.t. 18 h, then cooled to -78 °C and NaBH <sub>4</sub> added, 0 °C, 5h. <sup>95</sup>	Not desired product, <b>169</b>
4	TiCl(O <sup>i</sup> Pr) <sub>3</sub> , r.t. 2 days, then NaBH <sub>4</sub> (2 eq.) and succinic acid (2 eq.) added, r.t. 3 h.	No Product
5	TiCl(O <sup>i</sup> Pr) <sub>3</sub> , r.t. 2 days, then 1M borane (2 eq.) and succinic acid (2 eq.) added, r.t. 3 h. <sup>96</sup>	As Entry 3
6	TiCl(O <sup>i</sup> Pr) <sub>3</sub> , r.t. 10 min, then NaBH(OAc) <sub>3</sub> , r.t. 6 h <sup>95</sup>	Not desired product, <b>169</b>
7	TiCl(O <sup>i</sup> Pr) <sub>3</sub> , DCM, r.t. 18 h.	No imine formation.

**Table 8.5:** Attempted Reductive Amination Conditions.



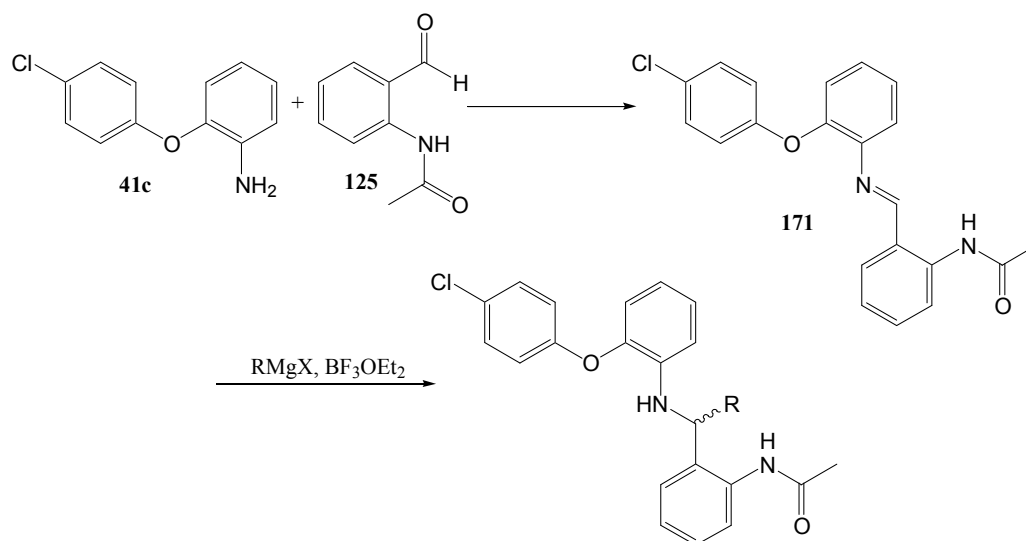
**Scheme 8.15:** Synthesis of **170** from **169**

So far it had not been possible to determine whether the problem with this reaction lay with the formation or reduction of the imine. It was therefore attempted to form and identify or, if possible, even isolate the intermediate imine. However, this was unsuccessful as no imine was formed. This proved that the problem lay with the formation of the imine,

probably due to the low reactivity of the ketone. This meant that a new synthetic route was required.

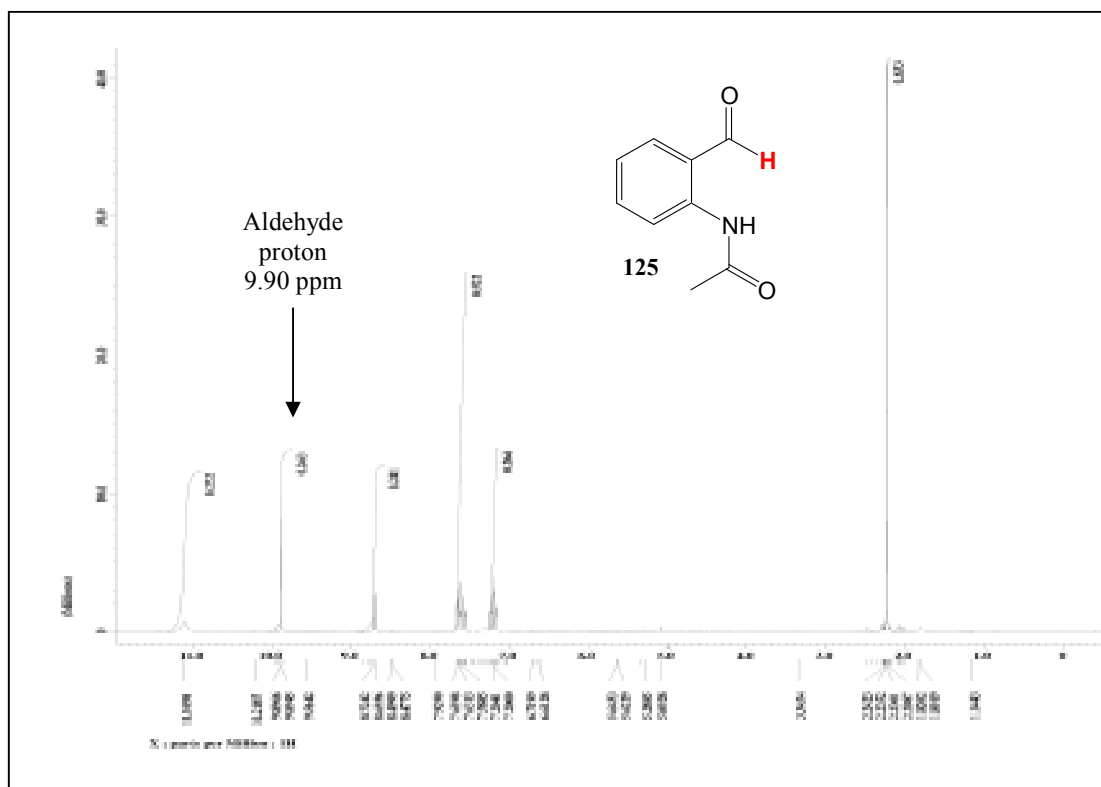
#### 8.4.6.3 Route B: Use of Organometallic Reagents.

It has been previously shown (Scheme 8.1) that the diphenylether aniline reacts successfully with the aldehyde, *N*-(2-formyl-phenyl)-acetamide, in a reductive amination to form compound **126**. It was hypothesised that if the intermediate imine (**171**) could be formed,<sup>95</sup> then organometallic chemistry techniques could be used to insert the desired alkyl groups into the imine bond, thus forming the products. The proposed synthetic route is shown in Scheme 8.16. The imine is formed and isolated, before being reacted with a Grignard reagent which will attack the imine and add the desired alkyl group. This route is ideal for this series of targets as the intermediates remain common until the last step, allowing for efficient diversification.

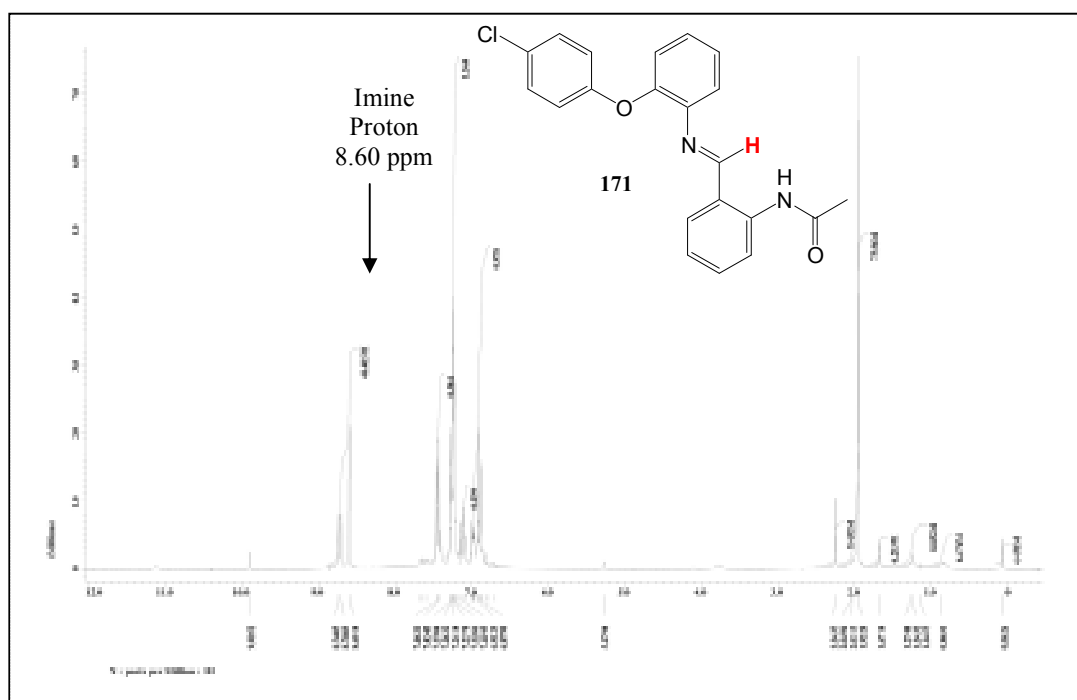


**Scheme 8.16:** Second proposed synthetic route to substituted targets

The formation of the imine intermediate (**171**) could be achieved using two different sets of conditions: either by stirring in DCM with 10 eq. of anhydrous  $\text{MgSO}_4$  for 18 h, or by stirring in DCM with 2 eq.  $\text{TiCl}(\text{O}^i\text{Pr})_3$  for 4 h.<sup>95</sup> Both sets of conditions were effective, but the second procedure was chosen as the procedure of choice as it showed better reproducibility and exhibited a shorter reaction time. The conversion of aldehyde (**125**) to imine (**171**) was easily detected by proton NMR (Figures 8.19 and 8.20), as there was a distinct difference in the chemical shift between the aldehyde proton at 9.90 ppm (Figure 8.19) in the starting material and the imine proton at 8.60 ppm (Figure 8.20).



**Figure 8.19:**  $^1\text{H}$  NMR spectrum for the aldehyde starting material **125**



**Figure 8.20:**  $^1\text{H}$  NMR spectrum for the imine intermediate **171**.

Once the imine had been formed, the DCM was removed under vacuum and replaced with THF. The Grignard reactions were carried out immediately, thus minimising any chance of degradation of the imine.  $\text{BF}_3 \cdot \text{OEt}_2$  has been shown to enhance the reaction and so this was used in the procedures. Many different Grignard reagents were tested (Table 8.6). However, the majority of these met with little success. The procedures where phenyl, isopropyl, cyclopropyl, vinyl or methyl magnesium Grignard were used all exhibited a lack of reactivity, as in all cases a significant proportion of the starting imine was isolated, even following an aqueous work-up and flash column purification. It was attempted to overcome this lack of reactivity using heat. The reaction with  $\text{MeMgBr}$  was heated under both conventional reflux conditions (Entry 7) and microwave conditions (Entry 6).<sup>97</sup> In both cases, the heating caused a decomposition of the starting imine, without producing any of the desired product. Instead the diphenylether aniline was the only compound isolated.

Some success was observed with the Grignard procedures as the reactions between the preformed imine and both allylmagnesium bromide and benzylmagnesium bromide were moderately successful (Entries 8 and 9). These were carried out under the same conditions as previously with  $\text{BF}_3 \cdot \text{OEt}_2$  added, and as previously, the reactions were stirred for 18 h at room temperature. The allyl substituted product was isolated in 57 % yield (**172**), and the benzyl substituted product in 8 % yield (**173**).

Entry	Grignard	Conditions	Result
1	$\text{PhMgBr}$	$\text{BF}_3 \cdot \text{OEt}_2$ , r.t. 18 h	Imine isolated
2	$\text{IsopropylMgCl}$	$\text{BF}_3 \cdot \text{OEt}_2$ , r.t. 18 h	Imine isolated
3	$\text{CyclopropylMgBr}$	$\text{BF}_3 \cdot \text{OEt}_2$ , r.t. 18 h	Imine isolated
4	$\text{VinylMgBr}$	$\text{BF}_3 \cdot \text{OEt}_2$ , r.t. 18 h	Imine isolated
5	$\text{MeMgBr}$	$\text{BF}_3 \cdot \text{OEt}_2$ , r.t. 18h	Imine isolated
6	$\text{MeMgBr}$	$\text{BF}_3 \cdot \text{OEt}_2$ , MW 100°C, 10min	Aniline SM isolated
7	$\text{MeMgBr}$	$\text{BF}_3 \cdot \text{OEt}_2$ , Reflux, 6h	Aniline SM isolated
8	$\text{AllylMgBr}$	$\text{BF}_3 \cdot \text{OEt}_2$ , r.t. 18h	57 % isolated yield ( <b>172</b> )
9	$\text{BnMgBr}$	$\text{BF}_3 \cdot \text{OEt}_2$ , r.t. 18h	8 % isolated yield ( <b>173</b> )

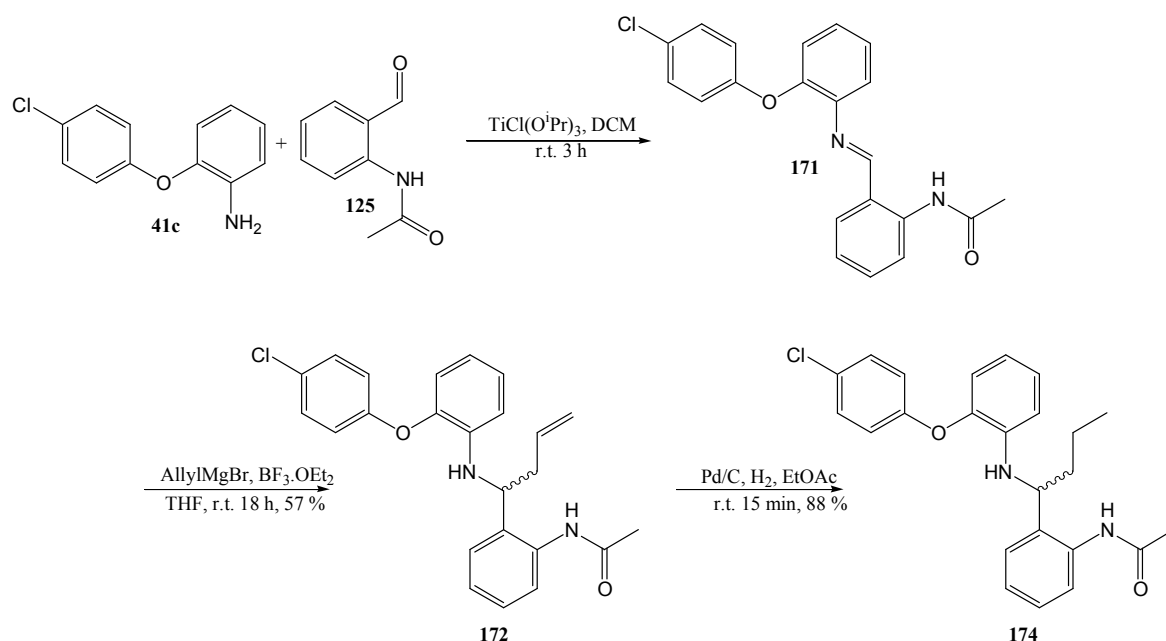
**Table 8.6:** Grignard Additions into Imine Intermediate.

It appears that there is a distinct difference between the reactivity of the allyl and benzyl Grignard reagents and the other Grignard reagents used. It is thought that this difference is



due to the basicity of the reagents. Organometallic reagents are strong bases and in this case it is possible that the reagents could therefore deprotonate the imine proton ( $pK_a \sim 22-24$ ) as well as the amide  $NH$ .<sup>98</sup> This would inactivate the Grignard, forming the corresponding alkane, though this can be compensated for by the usage of an excess of reagent (in this case 3 equivalents were used). However, the problem remains that the deprotonation of the imine means that it is no longer open to attack by the nucleophilic Grignard reagent. The imine stays in this deprotonated state until it is subjected to the aqueous work-up at the end of the reaction period, where the imine is reprotonated, regenerated and in some cases isolated. The allyl and benzyl Grignard reagents are much weaker bases so they are less likely to deprotonate the imine and therefore the imine is available to react with the nucleophilic Grignard reagent. The two products (allyl and benzyl) were only obtained in moderate yields. This may be due to the decomposition of the imine or due to some deprotonation still occurring, especially in the case of the benzyl Grignard.

The allyl substituted product is a useful intermediate, which could be successfully reduced to produce the propyl substituted compound (**174**, Scheme 8.17). This was achieved using palladium on carbon as the catalyst, in an atmosphere of hydrogen (atmospheric pressure). It was a fast reaction, reaching completion in just 15 min to produce compound **174**.



**Scheme 8.17:** Synthesis of compound **172** and its reduction to form compound **174**

At this stage there were three compounds in this series, the allyl, benzyl and propyl substituted compounds. However, a general route to a series of compounds had not been identified. For this reason, further investigations were made into the organometallic addition reactions (Table 8.7).

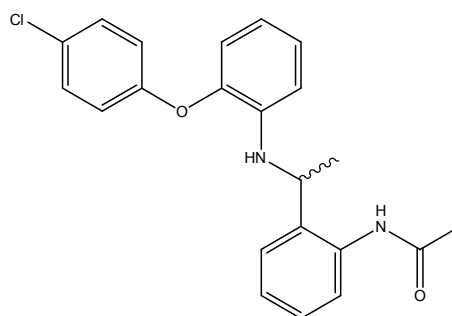
A review of recent literature revealed some possibly useful alterations to the Grignard reaction, with various other reagents added to alter the properties and reactivity of the reagents.<sup>99-104</sup> Unfortunately, the use of zinc chloride (Entry 1), copper iodide (Entry 2), scandium triflate (Entry 3) and the *N*-heterocyclic carbene, 1,3-bis(2,4,6-trimethylphenyl)-imidazolinium chloride (Entry 4) were unsuccessful and in most cases the imine was isolated.

The reagents used all attempt to alter the reactivity of the reaction in some way. For example, the use of copper iodide means that the copper will transmetallate the Grignard to give an organocopper reagent, which is softer in nature than the Grignard reagent.<sup>73</sup> This aimed to match the reactivity of the reagent to the imine bond, however it was not successful.

When cerium chloride was used as an additive, success was obtained (Entry 5) and the desired methyl substituted product was synthesised (**175**, Figure 8.21). It was hypothesised that the success observed using cerium chloride was due to the cerium chloride increasing the reactivity of the Grignard by decreasing the basicity and improving nucleophilicity. This further helps to further explain that the problem with this reaction, as mentioned previously, maybe due to the Grignard reagent acting as a base, deprotonating the imine and therefore stopping the desired reaction.

Entry	Conditions	Result
1	Isopropyl MgCl, <b>ZnCl<sub>2</sub></b> , r.t. 1 h, then imine added, r.t. 18 h <sup>99</sup>	Imine Isolated
2	Isopropyl MgCl, <b>CuI</b> , BF <sub>3</sub> OEt <sub>2</sub> , r.t. 18 h <sup>100</sup>	Imine Isolated
3	<b>Sc(OTf)<sub>3</sub></b> , Methyl MgBr, r.t. 18 h <sup>101</sup>	Imine Isolated
4	<b>1,3-Bis(2,4,6-trimethylphenyl)-imidazolinium chloride</b> , BuMgCl, r.t. 1 h, then imine, r.t. 3 days <sup>102</sup>	No Product
5	<b>CeCl<sub>3</sub></b> , MeMgBr, r.t. 2 h, then imine added, then r.t. 18 h <sup>103, 104</sup>	Correct Product isolated, <b>175</b> , 8%.

**Table 8.7:** Investigations into possible alternatives to the Grignard procedure



**Figure 8.21:** Compound **175**

However, the yield for the successful reaction was extremely low: just 8% isolated yield. For this reason, the reaction was repeated under the same conditions. However, the success could not be repeated and although the reaction was attempted many times no further product was isolated. The cerium chloride was purchased from Sigma-Aldrich as cerium chloride hexahydrate ( $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$ ) which requires dehydration to cerium chloride ( $\text{CeCl}_3$ ) before use. The original batch of cerium chloride hexahydrate had been dried in an oven before use, but this was not successful when repeated, so a few alternative methods were attempted. The cerium chloride was heated under vacuum for 5h, before use in the reaction, but with no success. In another attempt the cerium chloride was heated under vacuum as previously, and then left in an oven for 24h. None of the subsequent reactions showed any sign of product and unfortunately no explanation for this phenomenon was found so this reagent was abandoned. There was a sufficient amount of compound **175** synthesised to enable biological evaluation, but unfortunately the results have not been obtained as yet.

An alternative option to a Grignard reagent is to use an alkyl lithium reagent. The attempted procedures are shown in Table 8.8. However, similar problems with reactivity were encountered with the use of methyl lithium as with the methyl Grignard and only the starting imine could be isolated. A review of the chemical literature revealed that dimethylcopper lithium could be used as an alternative.<sup>105</sup> This was made *in-situ* using methyl lithium and copper iodide. However, this was also unsuccessful.

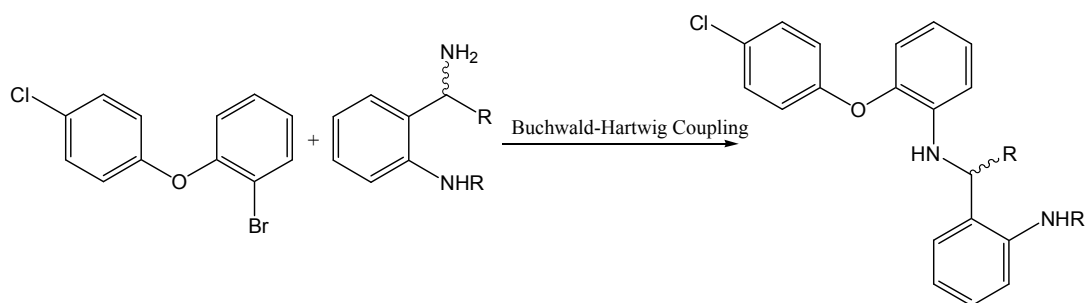
Entry	Conditions	Result
1	<b>MeLi</b> , -80 °C, 18h, r.t. 5 h <sup>106</sup>	Imine Isolated
2	<b>Me<sub>2</sub>CuLi</b> formed <i>in-situ</i> . CuI, MeLi, -40 °C, 1 h, then imine added, -40 °C to r.t. 18 h <sup>105</sup>	Imine Isolated

**Table 8.8:** Attempted procedures using organolithium reagents.

It was therefore decided that this route, reacting the intermediate imine with an organometallic reagent, was not a suitable route to this series of targets, as only the allyl substituted compound (**172**) could be synthesised successfully in moderate yields. A new route was required to enable a diverse range of these targets to be made successfully and, most importantly, reliably.

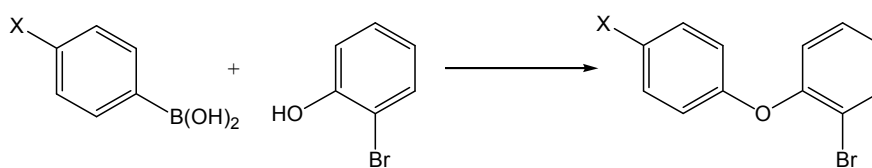
#### 8.4.6.4 Route C: Buchwald-Hartwig Amination.

Due to the lack of success so far, a whole new approach to this series of targets was required; a new way of forming the amine bond in the centre of the molecule was needed. One possible option was to use a Buchwald-Hartwig amination to couple the diphenylether headgroup to the rest of the molecule (Scheme 8.18).



**Scheme 8.18:** Third proposed route to the substituted targets

The synthesis of the diphenylether bromo intermediate has been previously reported. Nie *et al*<sup>107</sup> and Ricardson *et al*<sup>108</sup> both report the synthesis of a series of similar intermediates. The two methods vary in their choice of base (TEA and pyridine), but the yields are comparable. A comparison of these methods is shown in Table 8.9. It can be seen that the two methods both produce the desired products in moderate yields. In the case of the unsubstituted boronic acid the best yield of 47 % was obtained using TEA as the base<sup>107</sup>, after stirring at room temperature for 18h (Entry 1), and the yield did not increase with extended reaction times (Entry 2). Although, these yields were not ideal, the simplicity of the reaction and its purification allowed the chloro substituted bromo intermediate to be synthesised in the quantities required (Entry 4).

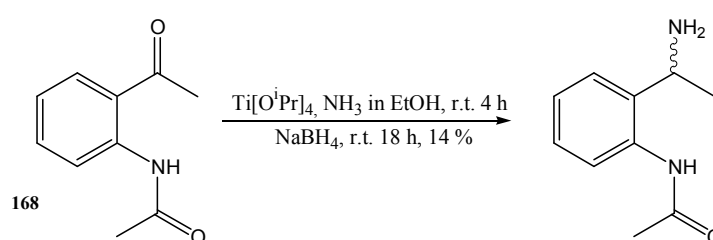


Entry	Compound no	Base used	Reaction time	X	Yield <sup>a</sup>
1	176	TEA <sup>107</sup>	18 h	H	47
2	176	TEA <sup>107</sup>	3 days	H	47
3	176	Pyridine <sup>108</sup>	3 days	H	36
4	177	TEA <sup>107</sup>	18 h	Cl	59

<sup>a</sup> Isolated yield.

**Table 8.9:** Comparison of methods for the synthesis of 1-bromo-2-phenoxy-benzene

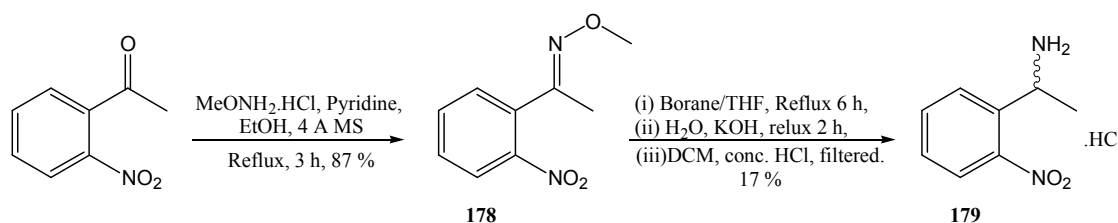
The synthesis of the other intermediate required for this route, *N*-[2-(1-amino-ethyl)-phenyl]-acetamide, was more problematic. It was initially attempted to convert the previously synthesised ketone *N*-(2-acetyl-phenyl)-acetamide into the desired primary amine (Scheme 8.19). Miriyala *et al*<sup>109</sup> reported a procedure for the conversion of carbonyl compounds to primary and secondary amines, using ammonia in ethanol solution and titanium isopropoxide to form the imine which was subsequently reduced by sodium borohydride. Unfortunately, the procedure was very problematic and showed only minimal success, although the product was at one stage isolated in a 14 % yield, this could not be repeated and no further product could be isolated. An alternative method using ammonium acetate<sup>110</sup> was attempted, but this was also unsuccessful.



**Scheme 8.19:** Synthesis of *N*-[2-(1-amino-ethyl)-phenyl]-acetamide.

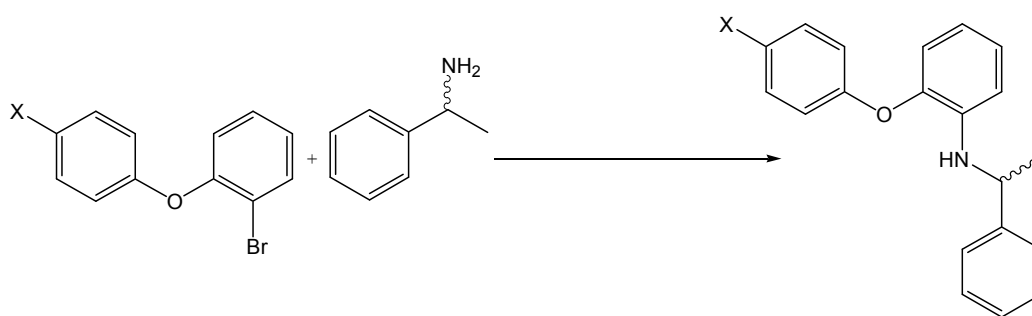
In case the *N*-acetyl group is able to interfere with this reaction in some way, the same procedure was attempted using 2-nitroacetophenone.<sup>109</sup> However, no product was isolated from the reaction, thus meaning a new approach to the synthesis of the primary amine intermediate was required.

A review of the chemical literature revealed an alternative method in which Salerno *et al* used an oxime as the intermediate in the synthesis of 1-(2-nitro-phenyl)-ethylamine **178**.<sup>111</sup> The oxime intermediate is more stable than other imines, because the methoxy group can participate in delocalisation of the imine double bond due to the alpha effect.<sup>73</sup> When this procedure was carried out it led to the successful synthesis of the desired product in moderate yields (Scheme 8.20). It should be noted that the primary amine is very hard to isolate, possibly due to instability to decomposition. For this reason, the amine was converted directly into the hydrochloride salt **179**, which could be isolated in a stable form. Although the overall yield for the synthesis of this product is poor, it is reproducible. It should also be noted that only a moderate yield of 50 % was reported by Salerno *et al*.<sup>111</sup>



**Scheme 8.20:** Synthesis of 1-(2-nitro-phenyl)-ethylamine

The next step in the synthetic procedure was the Buchwald-Hartwig amination with the previously synthesised bromo diphenylether headgroup. There are many different conditions for these types of reactions reported in the chemical literature.<sup>112</sup> In this particular case, the reaction conditions were investigated using the commercially available 1-phenylethylamine with the previously synthesised diphenylether bromo headgroup (Table 8.10). The first set of conditions (Entry 1) was unsuccessful and no product was obtained. The next set of conditions attempted was based on a paper by Harris *et al*,<sup>113</sup> detailing different conditions for the coupling of both primary and secondary amines and proved to be successful with the desired products obtained in modest yields (Entries 2 and 3).

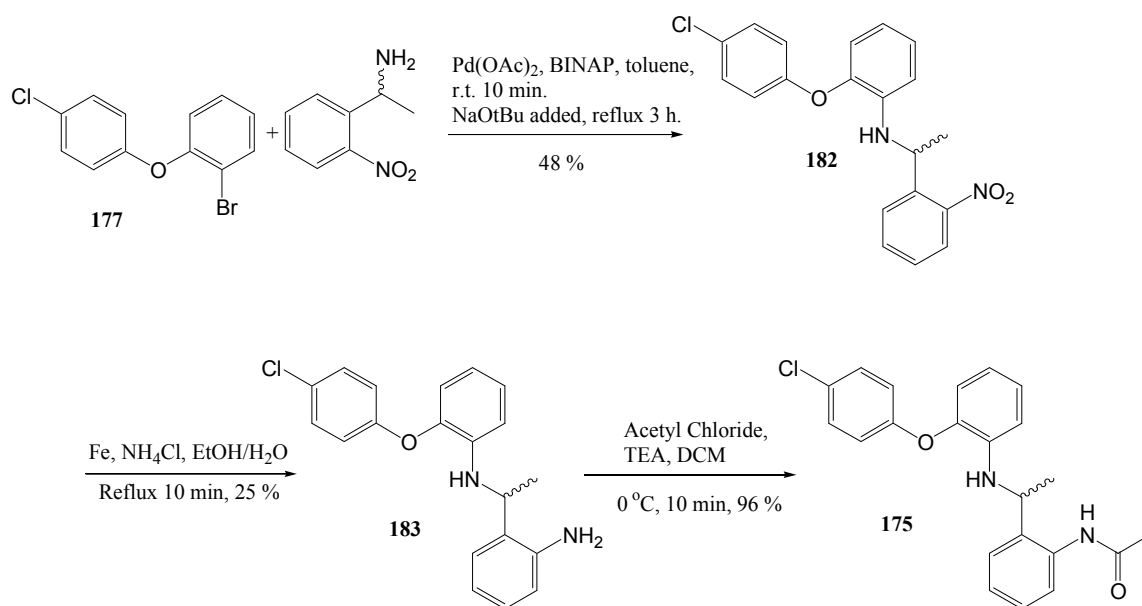


Compound no	Entry	X	Conditions	Yield <sup>a</sup>
180	1	H	Amine, Cs <sub>2</sub> CO <sub>3</sub> , Pd <sub>2</sub> (dba) <sub>3</sub> , BINAP, dioxane, sonicated 40 min. Bromo added, reflux 18 h.	No Product
180	2	H	Pd(OAc) <sub>2</sub> , BINAP, starting materials, toluene, r.t. 10 min. Then NaO <sup>t</sup> Bu added, reflux 18 h. <sup>113</sup>	49%
181	3	Cl	Pd(OAc) <sub>2</sub> , BINAP, starting materials, toluene, r.t. 10 min. Then NaO <sup>t</sup> Bu added, reflux 3 h. <sup>113</sup>	41%

<sup>a</sup> Isolated Yield.

**Table 8.10:** Development of conditions for the Buchwald-Hartwig amination.

These conditions were then successfully applied to the desired targets, thus allowing the methyl substituted target in this series to be successfully and reliably synthesised. The Buchwald amination proceeded smoothly and gave the desired product in a moderate yield (48 %). From here it was just 2 further steps to the final product (Scheme 8.21). The nitro group in this intermediate was reduced using the standard conditions and then the primary amine was subsequently acetylated to produce the desired compound. Importantly, this route has been proved to be reproducible. Overall, the yields in this synthetic route are moderate, although it should be possible for all the yields to be improved with further optimisation.



**Scheme 8.21:** Successful synthesis of compound 175

Currently this route has only been used to prepare the methyl substituted compound. However, this route is likely to be applicable to many other alkyl groups. If the ketone can be purchased or synthesised then it should be possible to convert it into the desired final target using this route.

In summary, six compounds have been synthesised in this series, the allyl, benzyl, propyl and methyl substituted compounds. These six compounds have been submitted for biological evaluation, but the results have not been obtained as yet. Once these results have been obtained a decision can then be made as to whether this series is worth pursuing. If the biological results are favourable and functionality around this region is beneficial to activity, then the synthesis of many more compounds will be required to extend the SAR in this series. With the potential for further optimisation the developed synthetic route (Scheme 8.21) will allow a wide range of targets to be prepared.

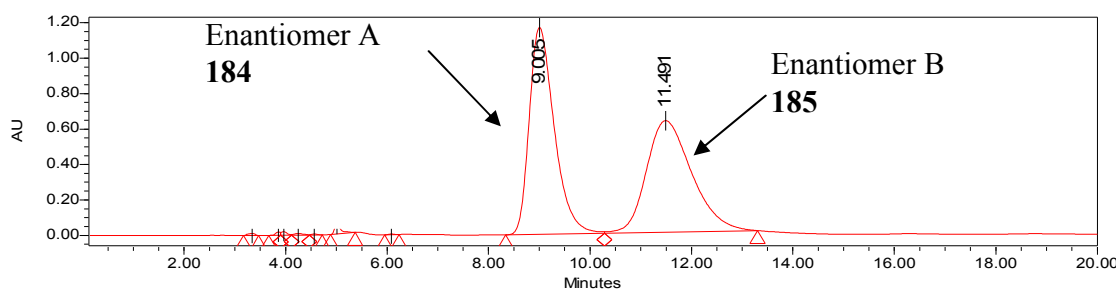
#### 8.4.6.5 Investigations into the Effects of Chirality on Enzyme Inhibition

The majority of the compounds prepared in this project so far have been achiral. This simplifies the SAR development process, as chirality can have a huge impact upon activity. So in order to get a good SAR each chiral form of a compound needs to be subjected to biological evaluation separately to establish if one or both enantiomers are active. Those compounds discussed in this Chapter, which are substituted on the amine CH<sub>2</sub> bond, are



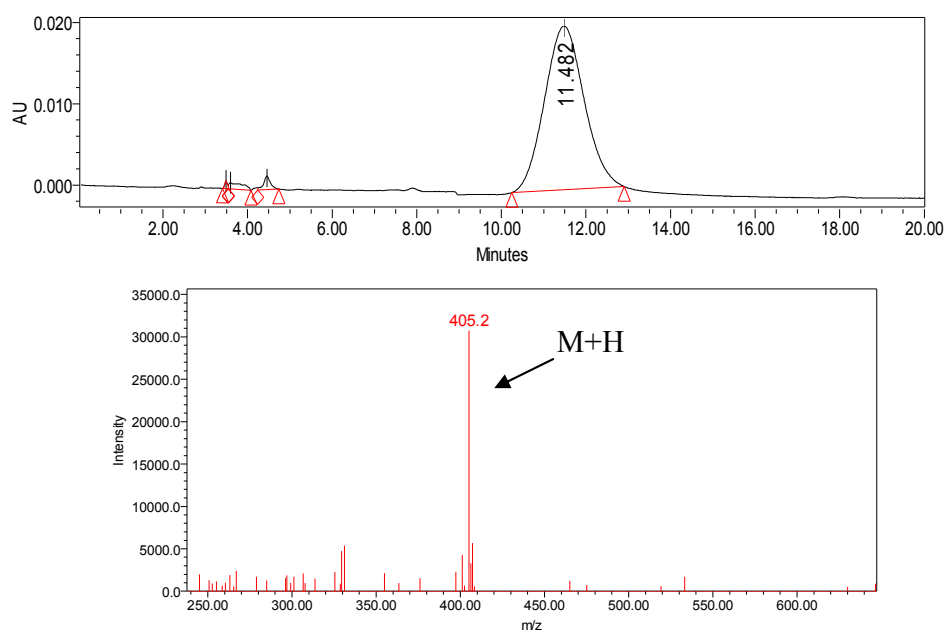
chiral compounds. They have one chiral centre in the molecule, therefore there are two possible enantiomers. It was therefore vital to look at the effects of chirality in this series, to assess the effects on the inhibitory activity of the compounds.

The most successful synthesis in this series so far was the synthesis of compound **172**, the allyl substituted compound (Scheme 8.17), which was prepared via an imine formation between the headgroup aniline and the corresponding aldehyde, which was then reacted with allylmagnesium bromide to form the desired product in a 57 % yield. This allowed a suitable amount of the compound (~ 200 mg) to be prepared. The product from this route is produced as a racemic mixture, with equal amounts of each enantiomer formed. Investigations were then conducted into how to separate the chiral forms of the compound. Preparative chiral HPLC can be a suitable option for separation of compounds on a small, laboratory scale. When compound **172** was subjected to separation using an analytical scale Chiralcel AD-H chiral HPLC column (Figure 8.22), it showed that the mixture contained, as expected, a 1:1 mixture of enantiomers. It was also clear that separation of the enantiomers was possible as the separation observed was good, with enantiomer A (**184**) having a retention time of 9.0 min and enantiomer B (**185**) 11.5 min.

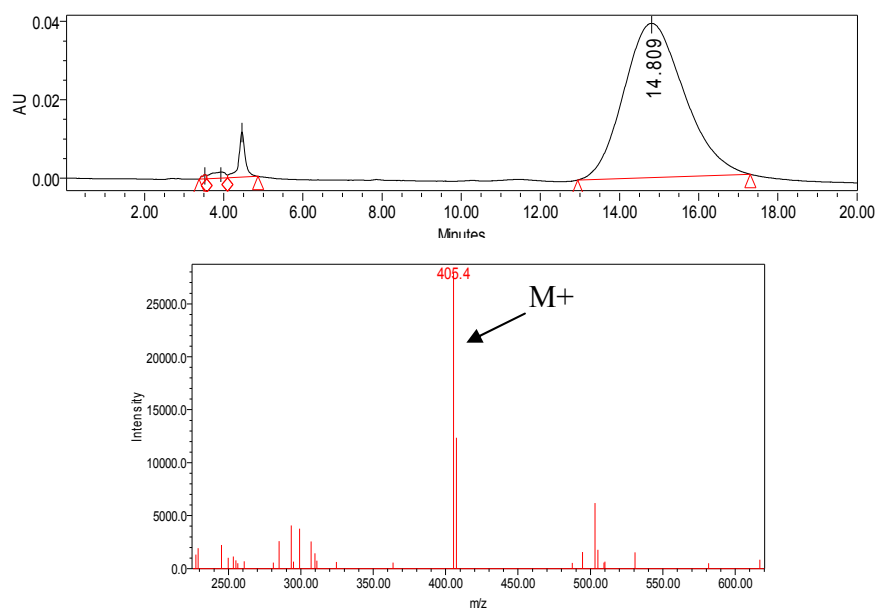


**Figure 8.22:** Chiral Separation of compound **172** using Chiralcel AD-H chiral HPLC Column (80 % methanol and 20 % water at 1.0 mL/min).

The compound was then subjected to separation on a preparative scale using the same column specification as before. This allowed ~ 70 mg of each compound to be isolated and analysed. It can be seen from Figures 8.23 and 8.24 that, once separated, enantiomers A and B were shown to be enantiomerically pure by the use of a Chiralcel column for the LCMS analysis.



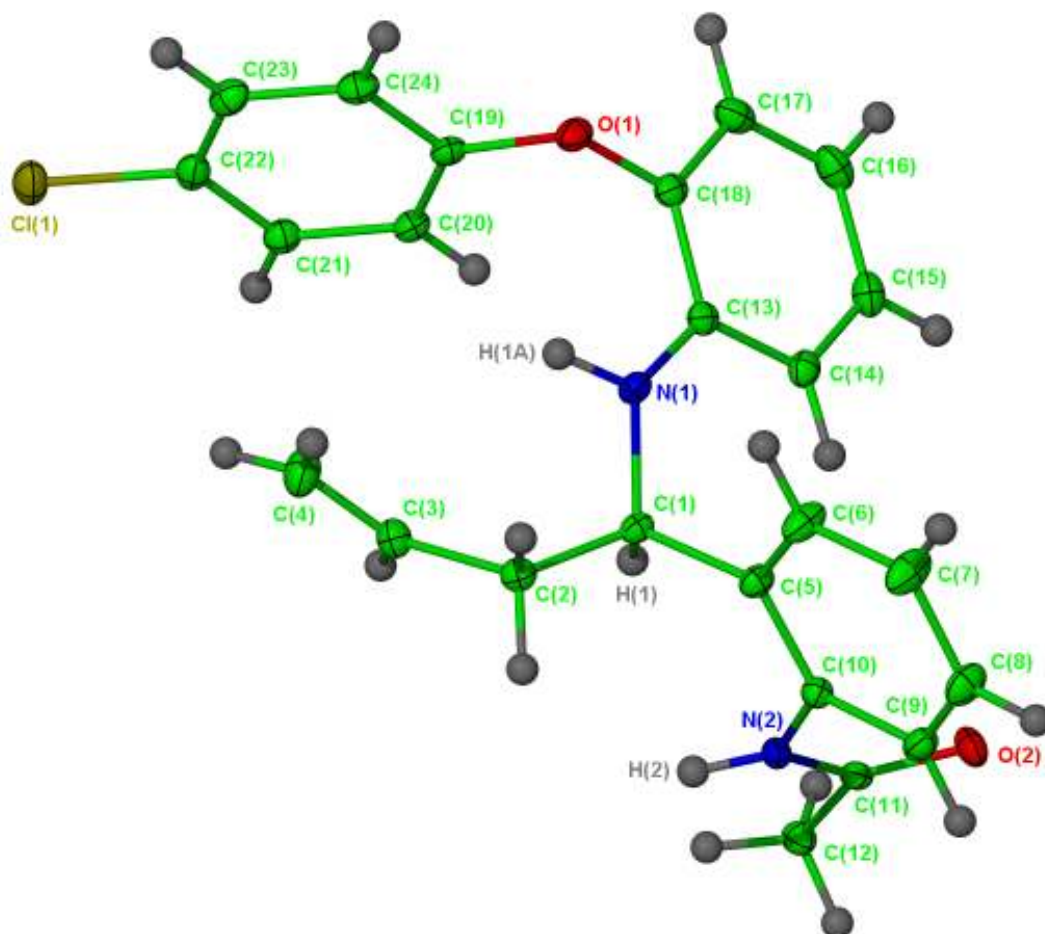
**Figure 8.23:** LCMS trace of Enantiomer A (**184**) of compound **172** using Chiralcel AD-H chiral column (80 % methanol, 20 % water at 1.2 mL/min).



**Figure 8.24:** LCMS trace of Enantiomer B (**185**) of compound **172** using Chiralcel AD-H chiral column (80 % methanol, 20 % water at 1.2 mL/min).

In order to identify the absolute stereochemistry of the enantiomers it was necessary to use X-ray crystallography. Both enantiomers were crystallised (from hexane/DCM) and enantiomer A was analysed by X-ray crystallography (Figures 8.25 and 8.26, see also

Appendix 3). This allowed enantiomer A to be conclusively identified as the *R*-enantiomer, meaning that enantiomer B is therefore the *S*-enantiomer.



**Figure 8.25:** X-Ray crystal structure of enantiomer A (**184**, (*R*)-).

It can be seen from Figure 8.26 that hydrogen bonds form between the hydrogen (grey, H2) on the acetamide nitrogen (blue, N2) and the carbonyl (red, O2) of a neighbouring molecule.

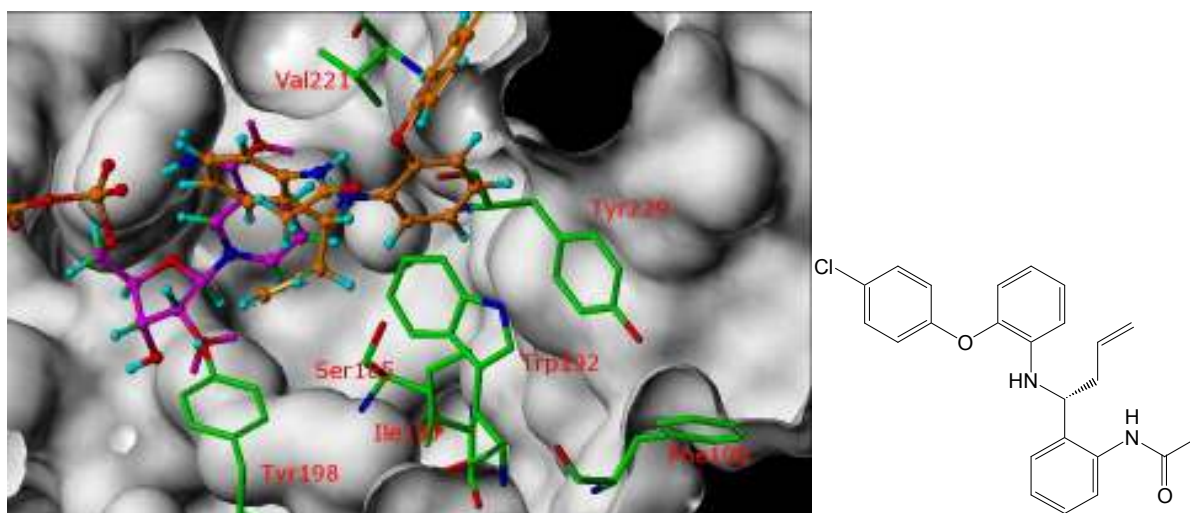


Once these two compounds had been separated and the absolute configurations had been determined they were both submitted for biological testing. The results are awaited.

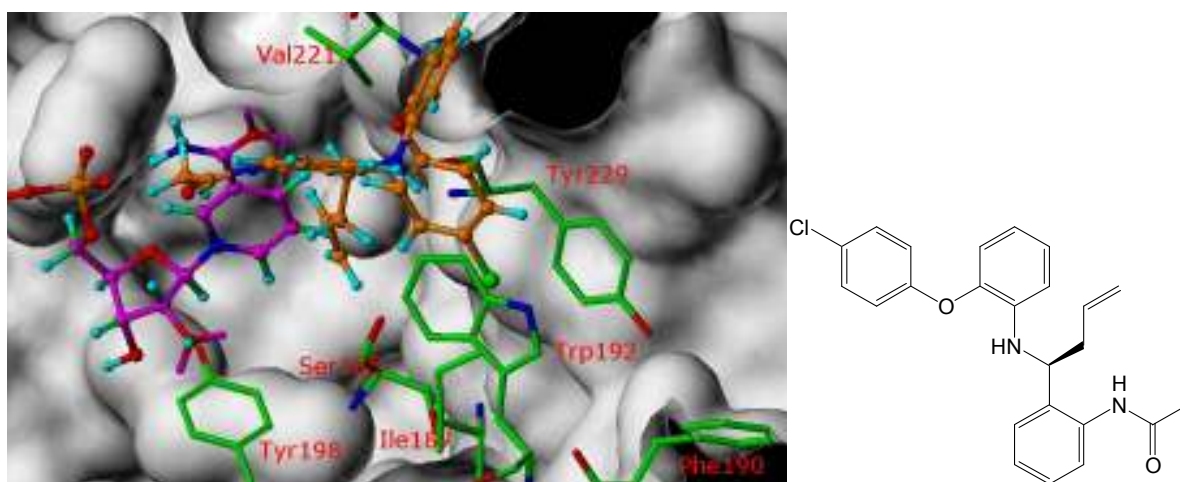
Both the racemic mixture and the two enantiomers will be evaluated, thus giving very important information about the active site. Biological systems show a degree of chirality, so it is possible that one enantiomer of compound **172** may be less active while the other more active than the racemic mixture. Whichever is the more active enantiomer is likely to be the active enantiomer across this substituted series of compounds.

Both enantiomers were docked into the 17 $\beta$ -HSD Type 3 homology model as shown in Figures 8.28 and 8.29. Both compounds docked into the model in multiple modes due to the high degree of flexibility from the high number of rotatable bonds (only the one mode is shown in Figures 8.28 and 8.29). Enantiomer A (**184**) has a predicted hydrogen bond between the carbonyl oxygen and Tyr198 side chain. In enantiomer B (**185**) a hydrogen bond is predicted between the diphenylether oxygen and a nitrogen atom in Val221 side chain.

Overall, the protein-ligand van der Waals interactions are highest for enantiomer A (**184**), indicating that this compound is a better fit into the active site. Therefore, it is anticipated that enantiomer A (**184**), the *R*-(-)- enantiomer, is likely to be the more effective inhibitor. This can only be proved when biological testing has taken place.

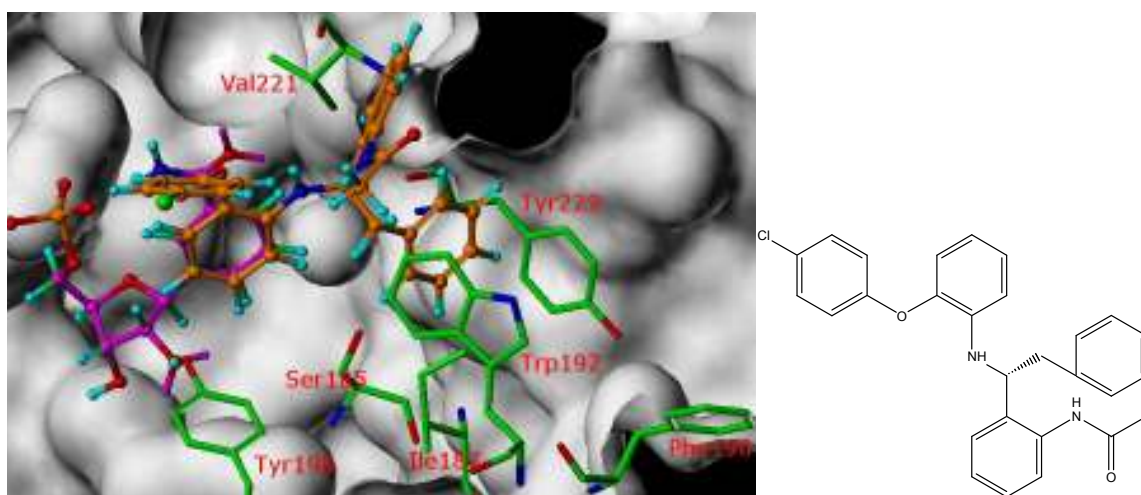


**Figure 8.28:** Enantiomer A (**184**, *R*-(-)-) docked into the 17 $\beta$ -HSD Type 3 homology model.



**Figure 8.29:** Enantiomer B (**185**, *S*-(+)-) docked into the 17 $\beta$ -HSD Type 3 homology model.

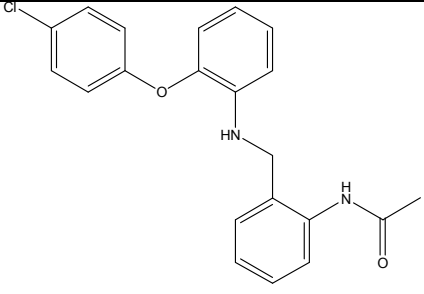
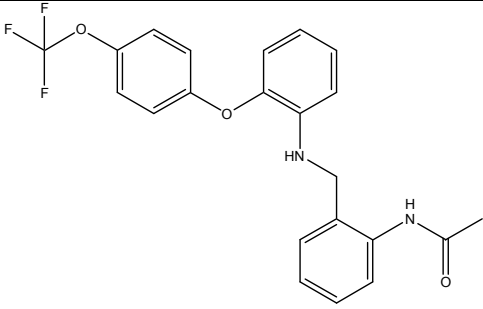
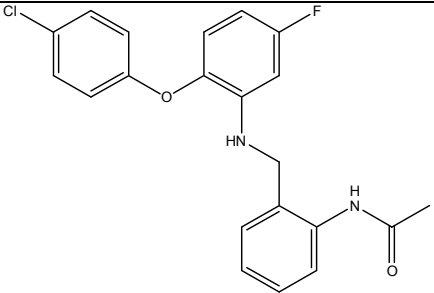
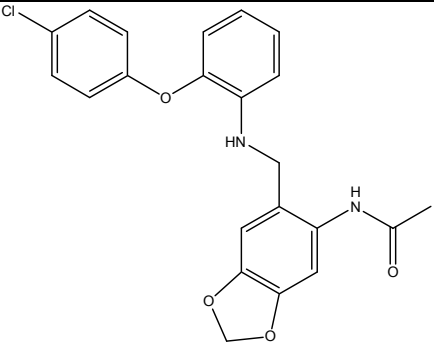
Compound **173** was also synthesised as a racemic mixture and has so far not undergone chiral separation. Each enantiomer of this compound was docked into the 17 $\beta$ -HSD Type 3 homology model to see if the same differences in docking between the enantiomers was repeated. Figure 8.30 shows the docking of the *R*-enantiomer into the homology model. As previously, due to the high number of rotatable bonds the compound docked with multiple modes, but in this case no potential hydrogen bonds were observed. Comparing the two enantiomers, the equivalent rings are placed in similar positions and there is possible  $\pi$ - $\pi$  stacking interaction between the compounds and the cofactor, although this is more optimal for the *R*- enantiomer. The protein–ligand van der Waals interactions are considerably higher for the *R*- enantiomer indicating that it is a better fit in the active site. Thus, as with the enantiomers of **172** the *R*- enantiomer is again predicted to be the more effective inhibitor.



**Figure 8.30:** *R*-Enantiomer of compound **173** docked into the 17 $\beta$ -HSD Type 3 homology model.

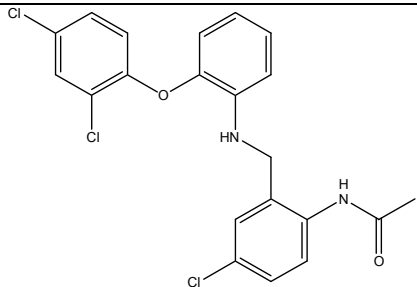
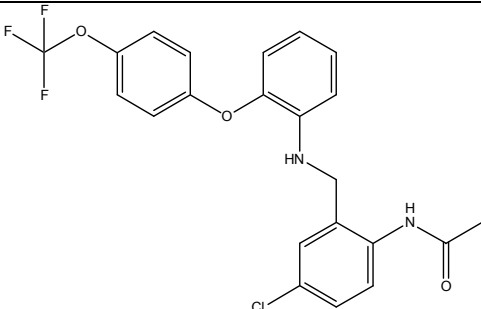
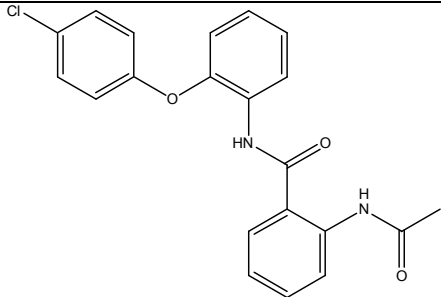
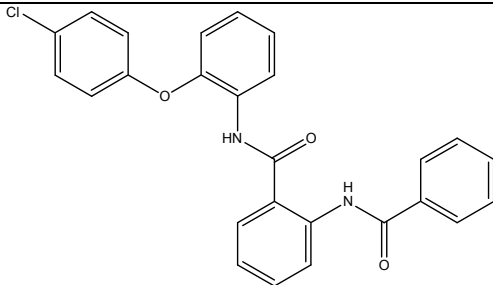
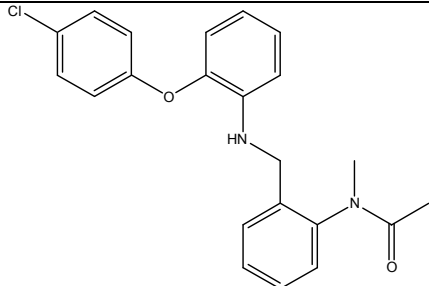
It is anticipated that, if the two enantiomers do show differing activity that all the biologically significant analogues prepared in this series would need to be separated into the corresponding enantiomers and subjected to further biological testing. When they are received the biological results will be very useful to the progression of the project. It is hoped that an asymmetric synthetic route could be developed for the synthesis of these compounds if one enantiomer is shown to be significantly more active than the other.

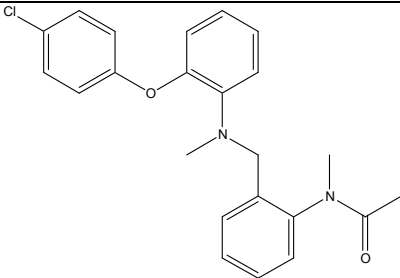
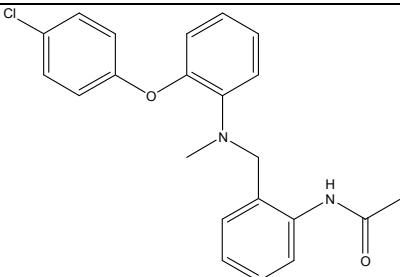
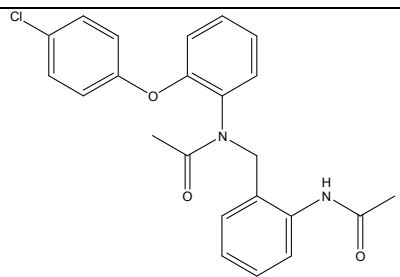
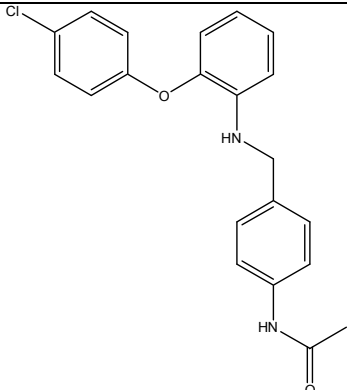
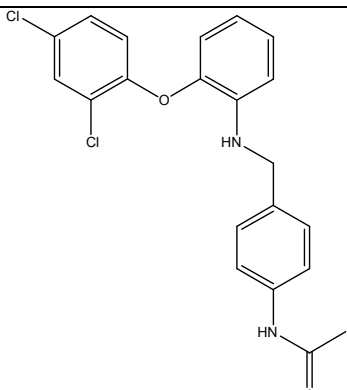
## 8.5 Table of Compounds Synthesised within Chapter 8

Compound no.	Structure	% Inhibition (at 10 $\mu$ M). <sup>a</sup>
126		87 $IC_{50} = 0.9 \mu M$
127		b
128		b
131		86

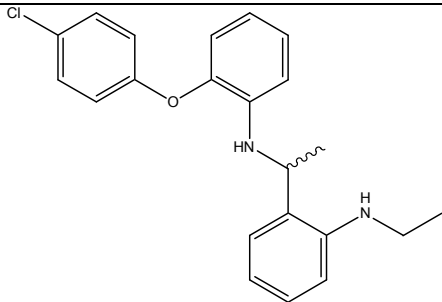
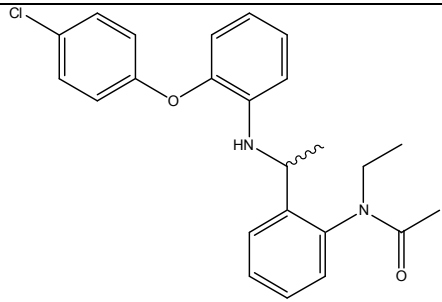
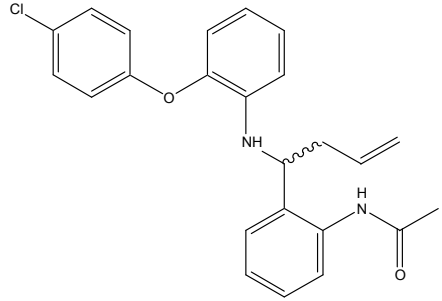
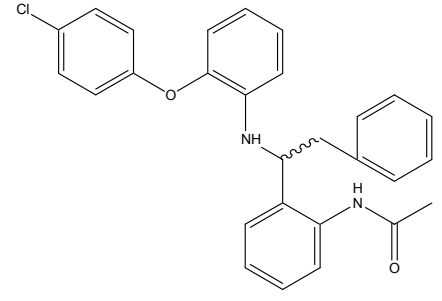
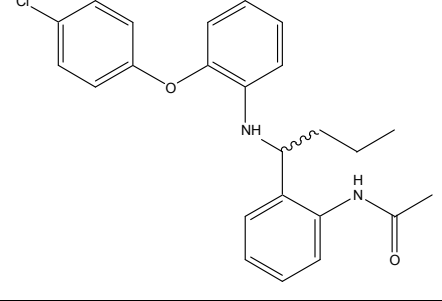


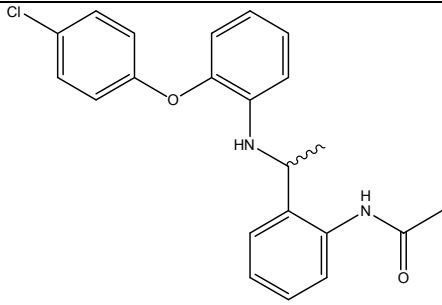
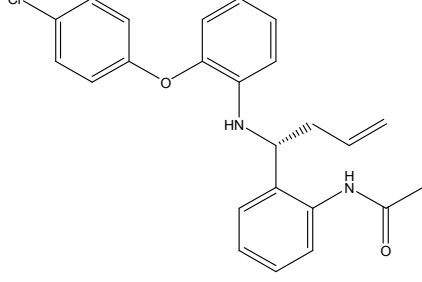
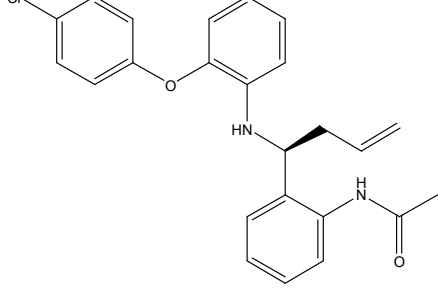
138		b
139		b
140		b
141		b
142		<p>97</p> <p>IC<sub>50</sub> = 1.9 μM</p>

143		b
144		b
147		b
148		b
150		b

151		b
152		b
153		b
154		29
155		b



169		b
170		b
172		b
173		b
174		b

175		b
184	 <b>R-(-)-</b>	b
185	 <b>S-(+)-</b>	b

<sup>a</sup> Results obtained from the TLC assay. Mean of at least 2 measurements with typically a SD of  $\pm 5\%$ , <sup>b</sup> Not determined.

## Chapter 9

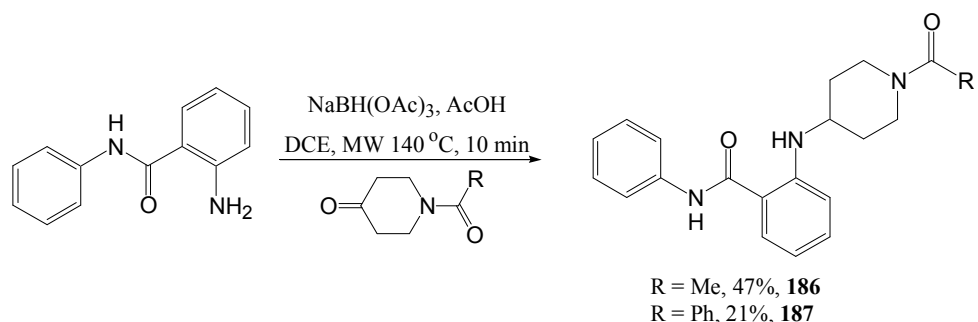
### Design and Synthesis of Compounds with an Amide Linked Hydrophobic Headgroup

#### 9.1 Synthesis of Initial Targets

All the compounds discussed so far have had the same diphenylether headgroup, albeit with differing substituents. Other areas of the target compounds have been varied greatly to explore the SAR with the 17 $\beta$ -HSD Type 3 enzyme, so it would be interesting now to introduce alternatives to the diphenylether hydrophobic headgroup in order to further probe the SAR.

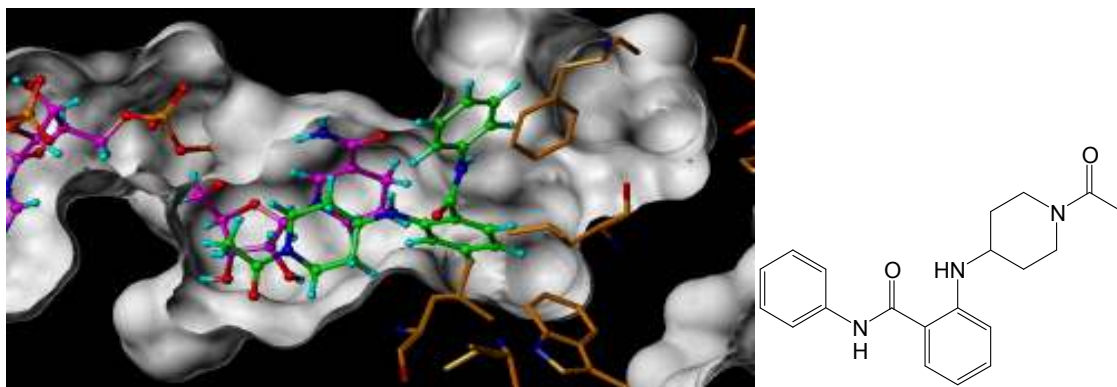
One variation that may prove to be interesting is the incorporation of an amide linking group between the two aryl groups of the hydrophobic head unit. It would be an interesting change in the size, geometry and electronic properties of the molecule, and therefore useful to the development of the SAR.

During the planning stages of this series, it was found that 2-aminobenzanilide was commercially available, meaning that the synthesis of compounds with an amide linked hydrophobic headgroup was possible in just one synthetic step (Scheme 9.1). The compounds were synthesised in one step from commercial starting materials using the standard reductive amination conditions discussed in Chapter 7.<sup>85</sup> The compounds (**186** and **187**) were obtained in moderate yields and have both been submitted for biological testing.



**Scheme 9.1:** Synthesis of compounds with an amide linked hydrophobic headgroup **186** and **187**.

Figure 9.1 shows compound **187** docked into the 17 $\beta$ -HSD Type 3 homology model. It can be seen from this docking that the compound lies in a similar alignment to previous compounds, with the hydrophobic headgroup interacting with Phe-214 (See Chapter 6) and the *N*-acetyl carbonyl group is positioned towards the cofactor. Until biological data has been obtained it is unknown if this similarity in alignment will be important to inhibitory activity.

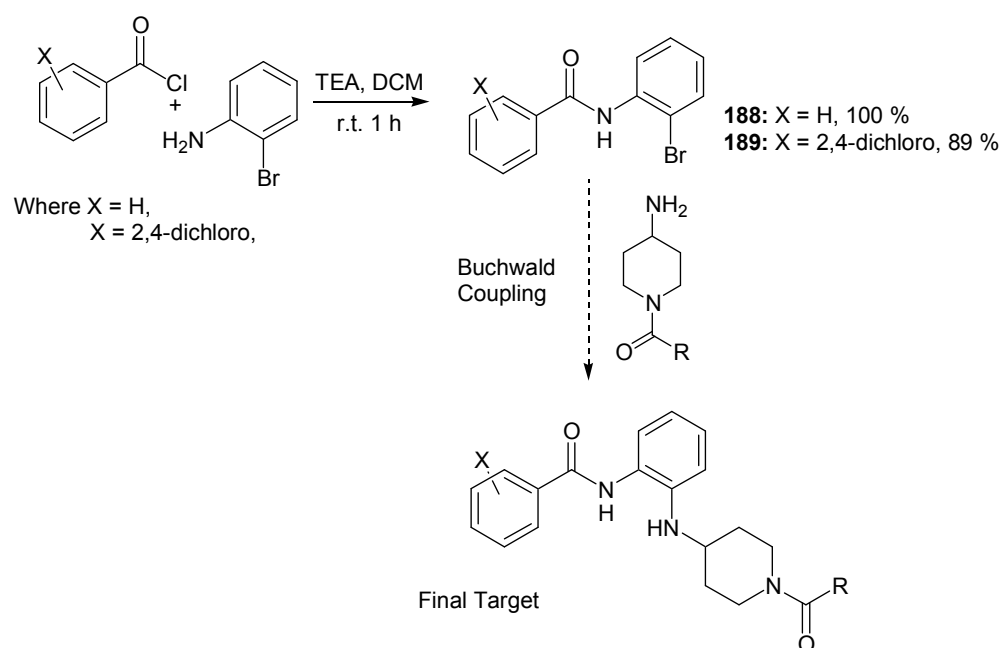


**Figure 9.1:** Compound **187** docked into the 17 $\beta$ -HSD Type 3 homology model.

Within this series of compounds with an amide linked hydrophobic headgroup it is possible for the amide to be in two different forms, the original form (shown in Scheme 9.1) and the reversed formation, where the CONH are reversed. There was no *in-silico* docking information or biological data to suggest that either form may be more active than the other, so both forms required synthesis and biological testing.

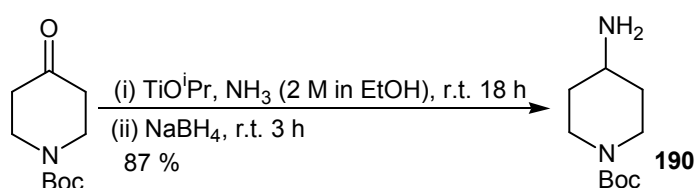
The route to the compounds with a reversed amide linked hydrophobic head group was initiated. There was not a suitable starting material commercially available, so in this case a different synthetic route was required. This new route was to utilise a Buchwald-Hartwig amination<sup>112, 114, 115</sup> to couple the bromo substituted amine linked headgroup with a primary amine on the piperidine (Scheme 9.2). Two analogues of the hydrophobic headgroup were synthesised using benzoyl chloride and 2,4-dichlorobenzoyl chloride (Scheme 9.2). The first step, a coupling of 2-aminobromobenzene with the desired benzoyl chloride, was successful and compounds **188** and **189** were synthesised in excellent yields. The final step to the desired targets was planned to be a palladium catalysed Buchwald-Hartwig amination, with 4-amino-piperidine-1-carboxylic acid tert-butyl ester **190**, to afford the final amide linked hydrophobic headgroup target compounds. However, the preparation of 4-amino-piperidine-1-carboxylic acid tert-butyl ester, **190**, was not straightforward.





**Scheme 9.2:** Synthetic route to amide linked hydrophobic head group.

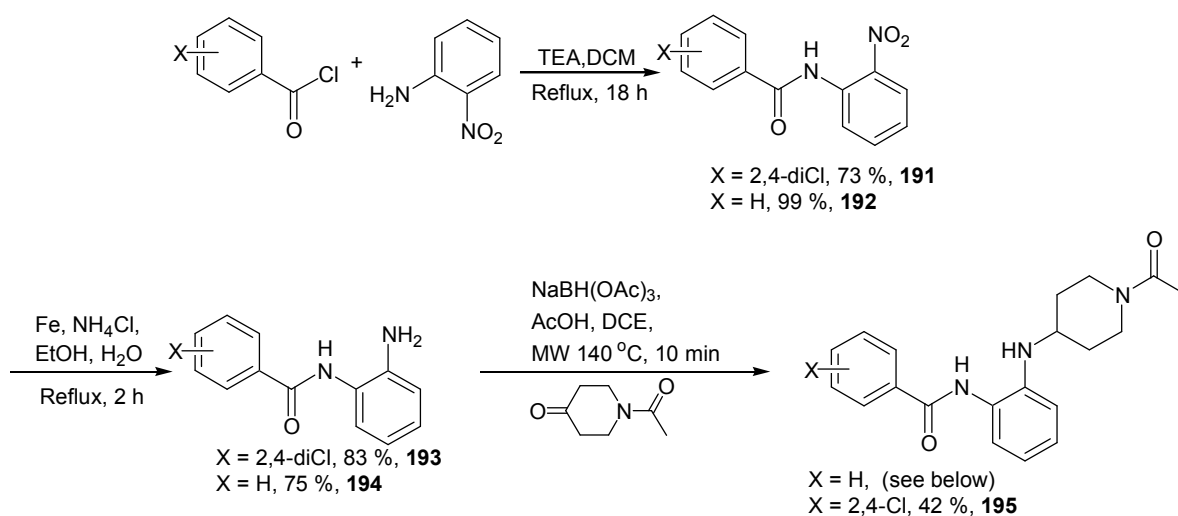
The synthesis of 4-amino-piperidine-1-carboxylic acid tert-butyl ester **190** was attempted using the method by Miriyala *et al*, previously mentioned in Chapter 8 (Scheme 9.3).<sup>109</sup> Firstly, the imine is formed using ammonia in ethanol solution with titanium *iso*-propoxide as the Lewis acid. This is subsequently reduced using sodium borohydride to form the desired primary amine. However, this reaction was very variable and unreliable. Some success was shown initially, but this success could not be repeated and, unfortunately therefore, this was not a viable route to the desired targets.



**Scheme 9.3:** Synthesis of 4-amino-piperidine-1-carboxylic acid tert-butyl ester.

The lack of synthetic success led to the planning and initiation of an alternative route to amine linked hydrophobic headgroup target compounds (Scheme 9.4). Formation of the amide bond in the headgroup was achieved, as previously, via an acid coupling, in this instance between a benzoyl chloride and 2-nitroaniline. This was achieved with excellent yields. The consequent reduction of the nitro group was also successful leading to the desired amide linked diphenyl aniline headgroup precursor (**193** and **194**). This was then

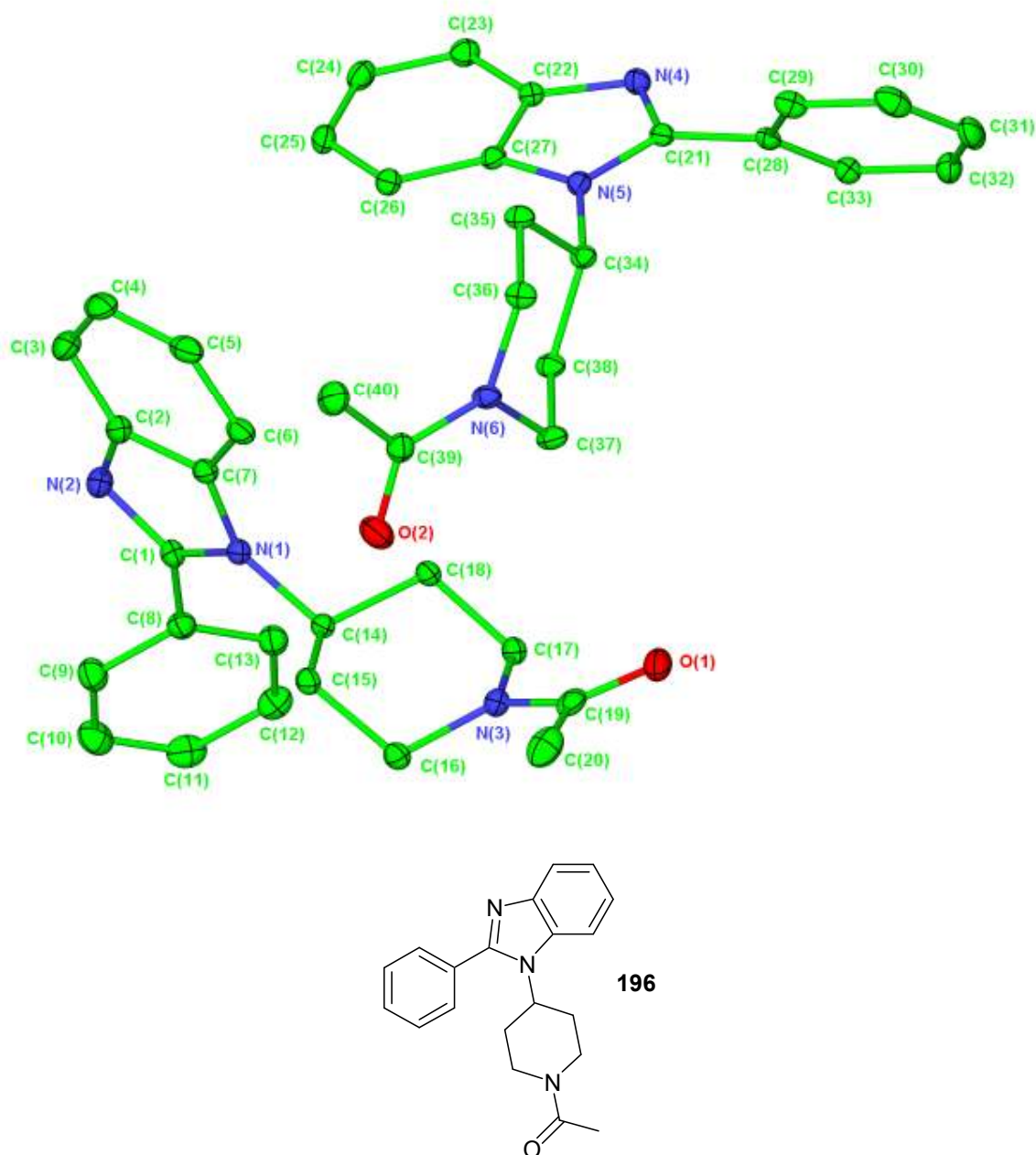
subjected to the normal reductive amination conditions described in Chapter 7.<sup>85</sup> In the case of the 2,4-dichloro substituted target, the reductive amination was successful and the first reversed amide linked hydrophobic headgroup target compound, **195**, was isolated in 42 % yield. This compound has been submitted for biological testing, but the results have not yet been obtained. However, in the case of the unsubstituted hydrophobic headgroup this route did not produce the desired compound, an alternative product was formed instead.



**Scheme 9.4:** The second and successful route to the amide linked hydrophobic headgroup.

## 9.2 The Formation of an Alternative Product

The products from the amide formation and nitro reduction were obtained in good yields using unsubstituted benzoyl chloride as the starting material, but the reductive amination did not produce the expected compound. The yield of the reaction was 72 %, a very good yield, thus proving that the compound identified was the main product from the reaction. Proton NMR, carbon NMR and LCMS data were used to try to identify the product. However, these were inconclusive, as although it could be proved that the product isolated was not as expected, it was not possible to categorically determine the structure of the product obtained. Crystalline material was obtained and thus X-ray crystallography was used to conclusively solve the structure. This is shown in Figure 9.2, (for full details see Appendix 3).

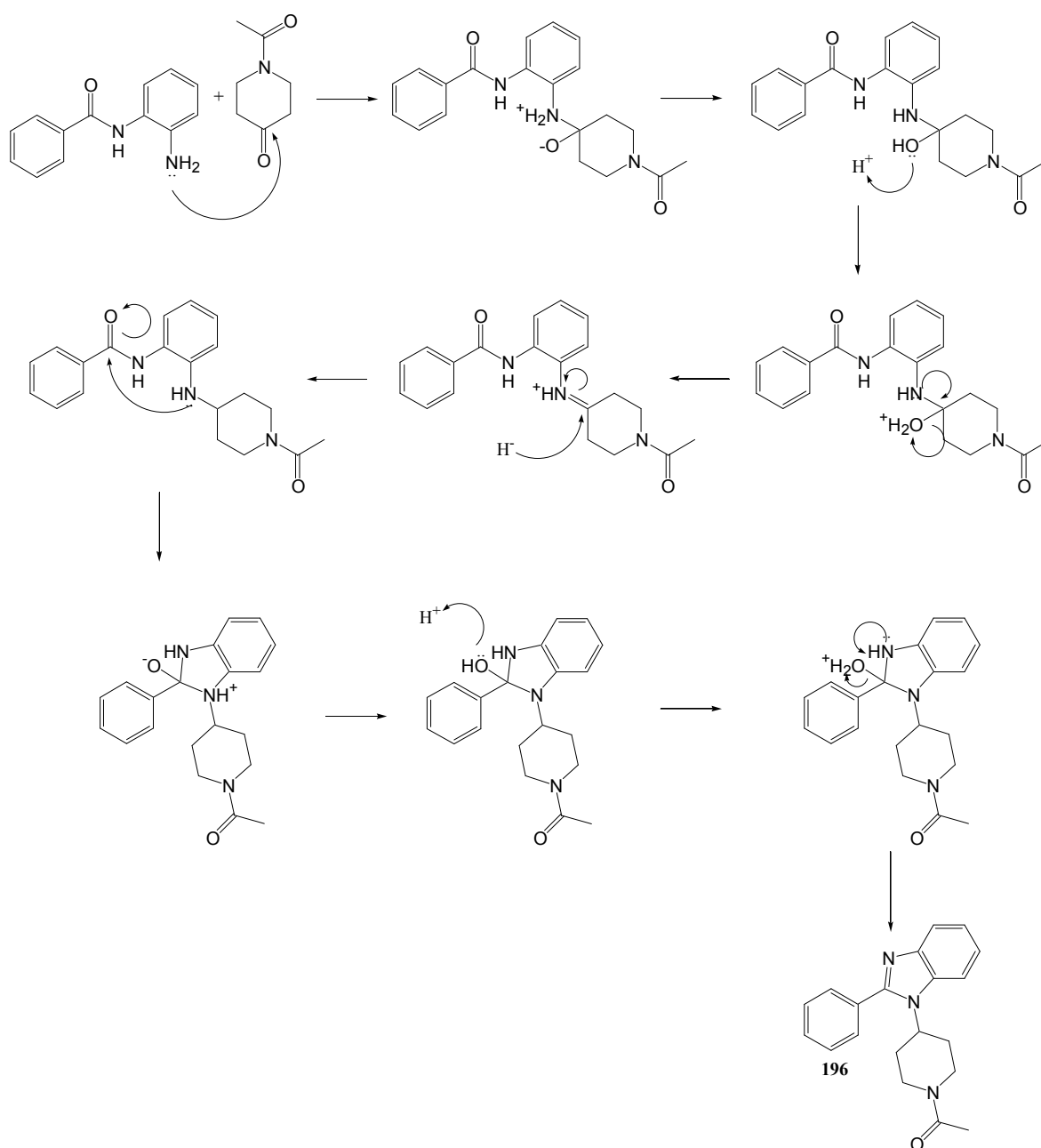


**Figure 9.2:** X-ray crystal structure of compound **196** and the confirmed structure

The X-ray crystal structure proved that, as hypothesised the reductive amination reaction between 1-acetyl-4-piperidone and *N*-(2-amino-phenyl)-benzamide did not produce the expected product, but an alternative benzimidazole product (**196**). Although compound **196** was not the desired compound, it still constitutes an interesting and novel hydrophobic head group within the project.

Figure 9.5 shows a proposed mechanism for the formation of compound **196**. Firstly, the starting materials undergo a reductive amination method, as expected, to form the desired product. However, at this stage the newly formed secondary amine undergoes an intramolecular reaction with the carbonyl group of the amide bond. This is an unexpected reaction, as the amide was assumed to be unreactive under these conditions. Following this attack and subsequent proton transfer, the imine is formed by electron transfer from the NH of the amide. The double bond is not reduced, so remains in the molecule, thus forming the final benzimidazole based compound. This reaction is driven by the intramolecular reactions leading to the formation of a stable aromatic product in the last step which is therefore irreversible. The use of MW irradiation is also presumably an influencing factor, as the energy available to the reactants is very high, thus assisting the reaction.

The presence of the 2,4-dichloro substitution is sufficient to inhibit this alternative reaction. The *ortho*-chlorine is likely to affect the electronic stability of the compounds and, in particular, the amide bond. The chloro substitutions may also affect the geometry of the compound, changing from the favourable geometry seemingly found in the unsubstituted intermediate to an unfavoured conformation, meaning that this alternative mechanism cannot occur.



**Scheme 9.5:** Proposed mechanism for the formation of compound **196**.

### 9.3 Synthesis of Extended Analogues

Using the information from the docking studies in Chapter 6 and 7, it is known that the active site of 17 $\beta$ -HSD Type 3 is very large. It would therefore be interesting to test the tolerability of the active site to large compounds. The synthesis of an extended analogue of **196** was therefore initiated (Scheme 9.6). The reductive amination between *N*-(2-amino-phenyl)-benzamide and 1-Boc-4-piperidine proceeded as previously in this series, with the



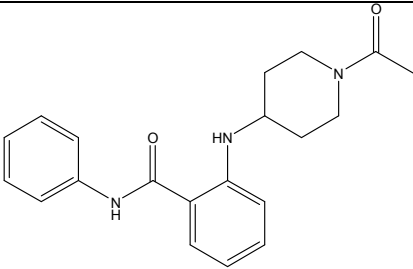
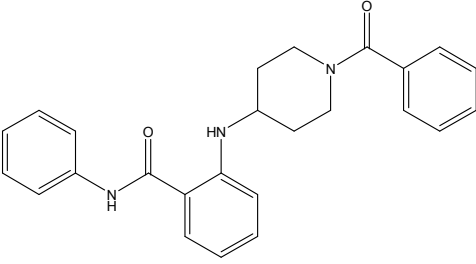
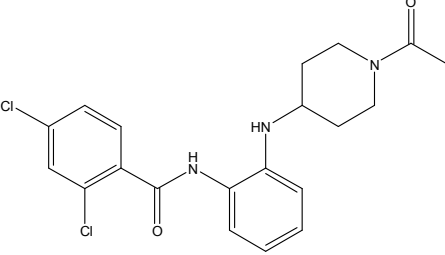
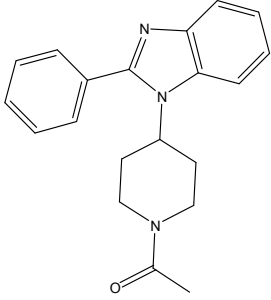
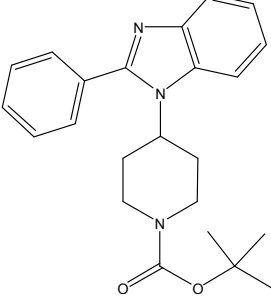
<b>Compound no</b>	<b>% Inhibition of 17<math>\beta</math>-HSD Type 3 (at 10 <math>\mu</math>M).<sup>a</sup></b>
<b>196</b>	13
<b>197</b>	<sup>b</sup>
<b>199</b>	<sup>b</sup>

<sup>a</sup> Results obtained from the TLC assay. Mean of at least 2 measurements with typically a SD of  $\pm 5$  %,

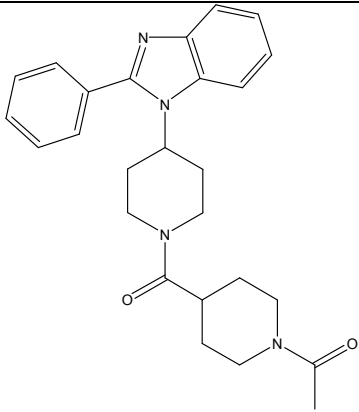
<sup>b</sup> Not determined.

**Table 9.1:** Biological results obtained for the compounds **196**, **197** and **199**.

### 9.5 Table of Compounds Synthesised within Chapter 9

Compound no.	Structure	% Inhibition (at 10 $\mu$ M). <sup>a</sup>
186		b
187		b
195		b
196		13
197		b



<p><b>199</b></p>		<p><sup>b</sup></p>
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<sup>a</sup> Results obtained from the TLC assay. Mean of at least 2 measurements with typically a SD of  $\pm 5\%$ ,

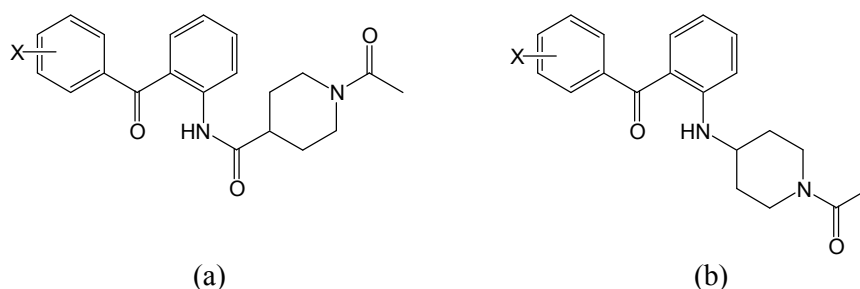
<sup>b</sup> Not determined.

## Chapter 10

### Design and Synthesis of Compounds with a Benzophenone Linked Headgroup

#### 10.1 Synthesis of Initial Targets

Another variation of the diphenylether hydrophobic headgroup that may prove interesting is the inclusion of a benzophenone headgroup (Figure 10.1). Although the compounds with a hydrophobic headgroup linked via an amine bond have, so far in the project, exhibited better inhibitory properties than the corresponding amide, it is possible that a change in another part of the molecule may alter this pattern. For this reason, synthesis of the benzophenone linked headgroup targets were planned in both the amide and amine linked series.



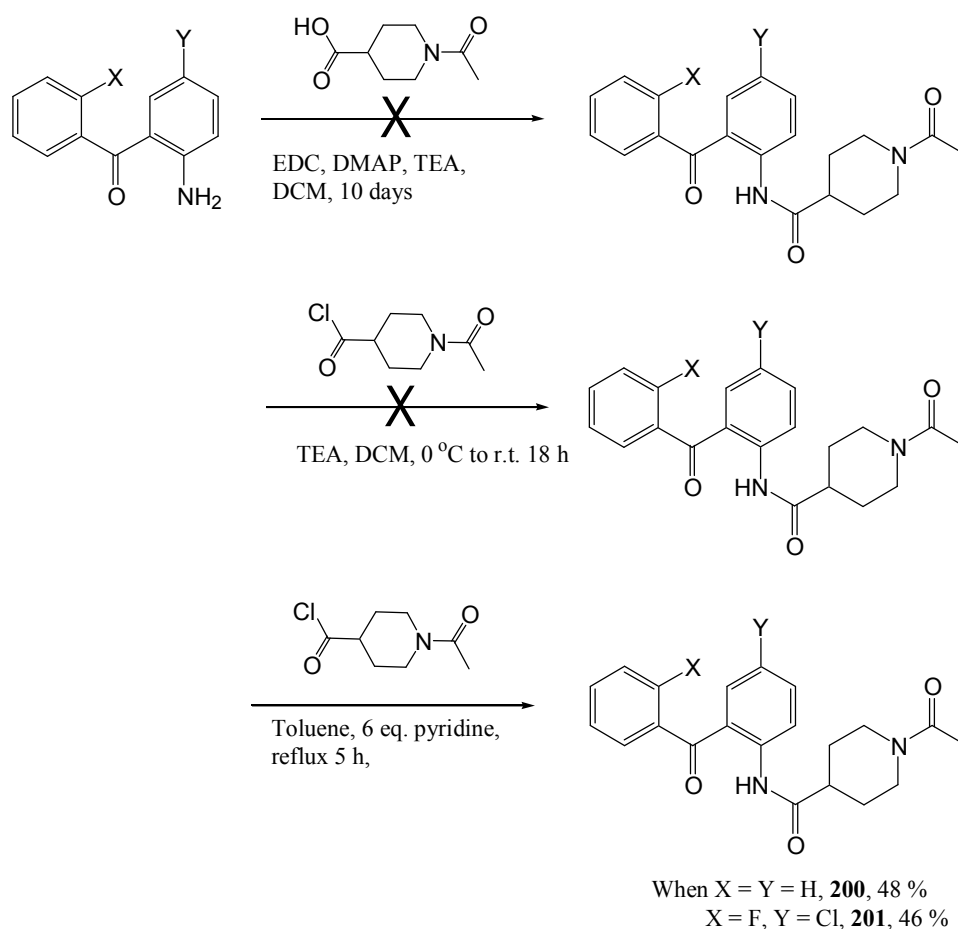
**Figure 10.1:** Initial targets in the benzophenone linked hydrophobic headgroup series.

The unsubstituted 2-aminobenzophenone and the halogen substituted 2-amino-5-chloro-2'-fluorobenzophenone were commercially available,<sup>88</sup> making the synthesis of these target compounds feasible in just one step.

##### 10.1.1 Amide Linked Targets

The synthesis of the amide linked targets in this series is shown in Scheme 10.1. Only one step was required from the 2-aminobenzophenone starting material to the final product. However, there was a problem encountered with the reactivity in this series. An amide coupling reaction was attempted, with 1-acetyl-piperidine-4-carboxylic acid, using EDC as the activating reagent. However, this was unsuccessful. Next it was attempted to form the amide bond using a standard reaction with the corresponding acid chloride. However, the same reactivity problem was encountered and only starting materials were isolated. A study of the chemical literature revealed two articles that used different, more forcing,

conditions to those already attempted. Park *et al*<sup>116</sup> heated the starting materials in DCM with 6 eq. pyridine for 6 h and Kettlera *et al*<sup>117</sup> used a very similar method, heating the reaction in toluene for 6 h. These two sets of conditions were combined and the desired reaction was heated in toluene with 6 eq. pyridine for 6 h. This produced the correct products in yields of 46 and 48 % respectively. The two compounds synthesised (**200** and **201**) have been submitted for biological evaluation, but the results have not yet been obtained.

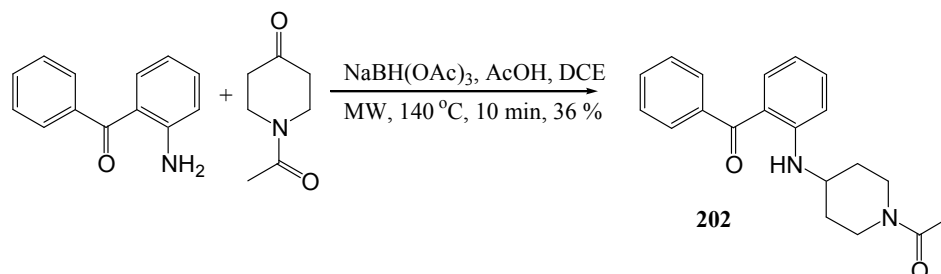


**Scheme 10.1:** Synthesis of amide linked compounds (**200** and **201**) with a benzophenone linked headgroup.

### 10.1.2 Amine Linked Targets and the Formation of Alternative Products

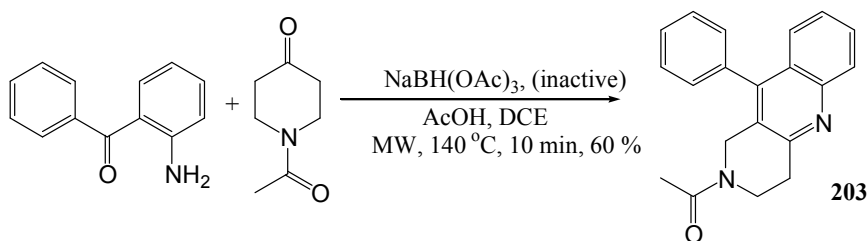
The synthesis of the amine linked compounds in this series was carried out using the standard MW reductive amination method detailed in Chapter 7 (Scheme 10.2).<sup>85</sup> Disappointingly, the yield was low at only 36 %, but this did mean that a sufficient amount

of compound **202** was synthesised for biological evaluation, but the result has not been obtained.



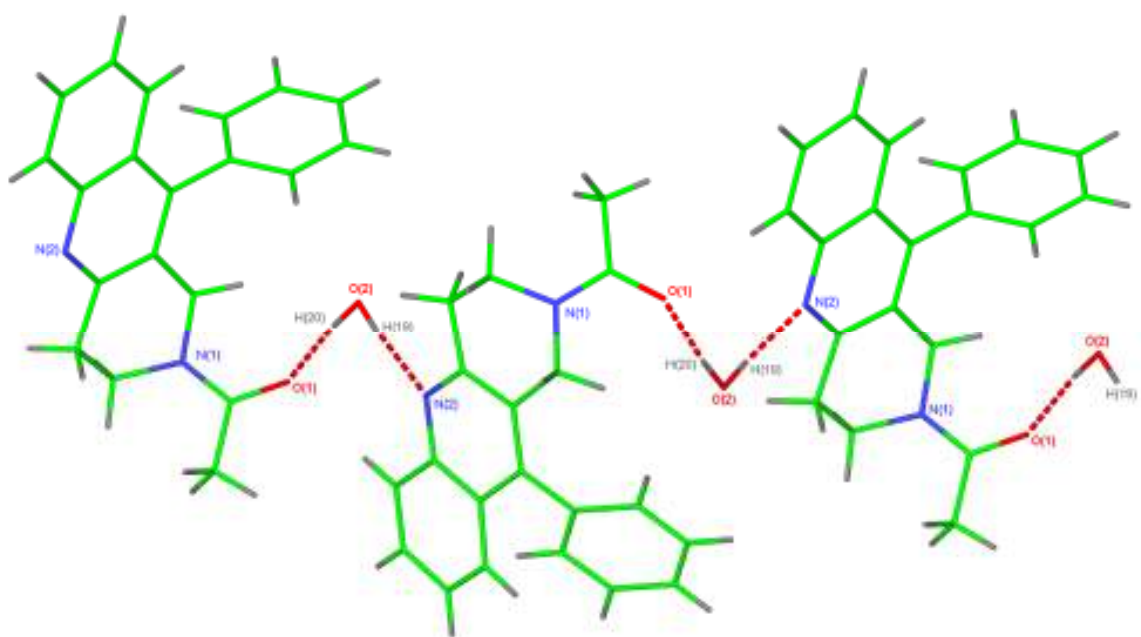
**Scheme 10.2:** Synthesis of amine linked compound **202**, with a benzophenone linked headgroup.

It was hoped that the yield of the above reaction (Scheme 10.2) could be improved from 36 %, so the reaction was repeated. However, a different product was obtained, in 60 % yield. Proton NMR, carbon NMR and LCMS data were used to try to identify the unexpected product. Crystalline material was obtained and X-ray crystallography was employed to confirm the structure of the product **203**, (Figures 10.2 and 10.3 and Appendix 3). It was found that the difference between the two reactions was the supply of  $\text{NaBH(OAc)}_3$  used. The  $\text{NaBH(OAc)}_3$  used in the second attempt was found to have degraded, and the alternative product had been formed in the absence of active  $\text{NaBH(OAc)}_3$ . Thus, by accident an interesting, novel modification of the Friedländer Synthesis of Quinolines has been discovered.<sup>118</sup>



**Scheme 10.3:** Formation of the alternative product **203**.



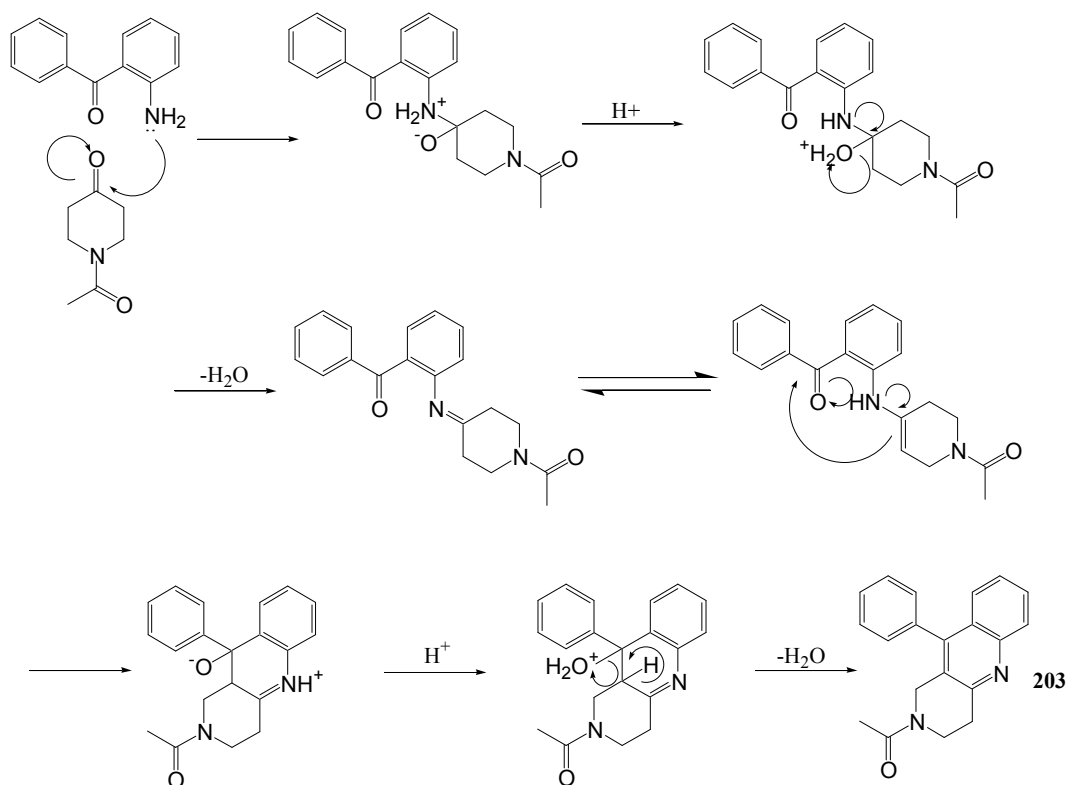


**Figure 10.3:** The packing structure of compound **203**

## 10.2 Optimisation of the Friedländer Synthesis of Quinolines.

Synthesis of the quinoline ring system is very important to the synthetic organic chemist, as it appears in many natural products and pharmaceutical entities, notably Quinine and Streptonigrin, an antibiotic. The structural core of quinolines can be made by many different methods,<sup>119</sup> including the Friedländer synthesis. The Friedländer synthesis of quinolones was originally published in 1882.<sup>118</sup> It is traditionally thought of as a reaction in which an *o*-aminobenzaldehyde is cyclised by reaction with a  $\alpha$ -methylene ketone in the presence of a base.<sup>120</sup> It has been shown that the Friedländer synthesis can be acid or base catalysed or it can even proceed without catalysis although uncatalysed Friedländer synthesis requires very high temperatures, up to 220 °C.<sup>119</sup> In many cases, it has been found that acid catalysis is more effective than basis catalysis.<sup>121</sup> Catalysts used for this reaction include hydrochloric acid,<sup>121</sup> sulfamic acid,<sup>122</sup> CuCl<sub>2</sub>,<sup>123</sup> *p*-toluenesulphonic acid,<sup>124</sup> chlorotrimethylsilane,<sup>125</sup> and diphenylphosphate (DPP)<sup>126</sup> amongst others.<sup>119</sup> There has also been a recent flurry of MW enhanced procedures,<sup>121, 124-126</sup> which demonstrates the advantages that MW assisted synthesis have upon this reaction, in both speeding up the reaction and improving yields.<sup>127</sup> In summary, the Friedländer synthesis is a versatile and reliable reaction, with many different options depending on the substrates available.

A suggested mechanism of this reaction is shown in Scheme 10.4.<sup>119</sup> It is generally accepted that the reaction proceeds via the initial formation of a Schiff base, followed by an internal aldol condensation (Scheme 10.4).<sup>119</sup>

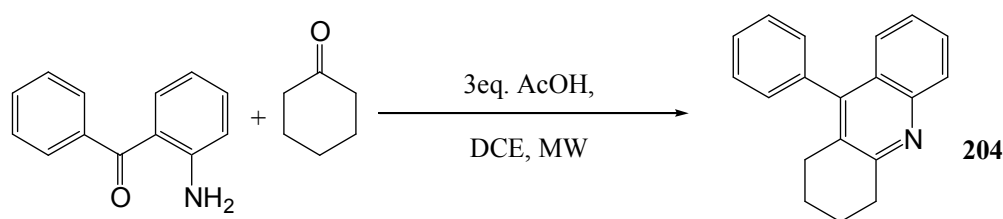


**Scheme 10.4:** Suggested mechanism for the formation of compound **203**.<sup>119</sup>

Many of the reactions in the literature show almost quantitative yields. This is an improvement over the 60 % yield exhibited so far under the conditions shown in Scheme 10.3, thus meaning that the yields of the reaction may be improved by optimisation. After the initial discovery of this alternative reaction, many investigations were made into the optimum conditions for this reaction. The reaction was repeated as previously (Scheme 10.3) except without the inclusion of  $\text{NaBH}(\text{OAc})_3$ , and the yield remained similar at 70 %.

The reaction between 2-aminobenzophenone and cyclohexanone was used to optimise the conditions. A study into the effects of time and temperature was initiated. The results are shown in Table 10.1. A solution of 2-aminobenzophenone (1 mmol), cyclohexanone (2 mmol) in DCE (2 mL) with AcOH (3 mmol) was heated as stated in Table 10.1. At room temperature the reaction does proceed, but incredibly slowly, with only an 18 % yield isolated after 3 days under standard conditions (r.t.). The yield was dramatically increased

by the use of MW technology (See Chapter 7 and Entries 1-6). It can be seen that an increased temperature leads to an increase in yields. However, above 200 °C there is no longer a significant increase observed (Entry 6) and higher temperatures could lead to degradation of starting materials or products. For this reason, 180 °C was the maximum temperature investigated from here onwards. It can also be stated that the optimum reaction time is a maximum of 20 minutes, as completion was not reached after 10 minutes and greater reaction times lead to reduced yields (Entry 2).



Entry	Temperature (MW heating, °C)	Time (min)	Yield (%) <sup>a</sup>
1	140	20	70
2	140	40	15
3	160	20	71
<b>4</b>	<b>180</b>	<b>20</b>	<b>78</b>
5	200	20	86
6	220	20	85

<sup>a</sup> Isolated yield.

**Table 10.1:** The effects of time and temperature on the Friedländer synthesis of quinolines, with 2-amino benzophenone and cyclohexanone.

As with the original MW reductive amination optimisation (Chapter 7), it was crucial to investigate an array of solvents that possess different microwave properties (Table 10.2).<sup>83, 86</sup> It can be seen that the use of acetonitrile does not greatly affect the yields, compared to DCE (Entries 2 and 3), whereas the use of toluene increases the yield from 78 % to 89 % (Entries 1 and 5).



Entry	Solvent	Temperature (MW heating, °C)	Time (min)	Yield (%) <sup>a</sup>
1	DCE	180	20	78
2	acetonitrile	180	20	71
3	Toluene	140	20	78
4	Toluene	160	20	83
<b>5</b>	<b>Toluene</b>	<b>180</b>	<b>20</b>	<b>89</b>
6	Toluene	180	10	72

<sup>a</sup> Isolated yield.

**Table 10.2:** The effects of solvent on the Friedländer synthesis of quinolones, with 2-amino benzophenone and cyclohexanone.

At this point, investigations were undertaken to attempt to drive the reaction fully to completion, by assisting with the dehydration (Scheme 10.4). It was hoped that the removal of water would help to encourage the forward equilibrium and therefore increase the yield of the reaction. Dehydrated magnesium sulphate (3 eq.) and 4 Å molecular sieves were tested, but reduced the isolated yield to 76 and 54 % respectively.

Investigations were also made to see whether the acetic acid used so far could be successfully substituted for alternative reagents (Table 10.3). These results show that, as expected, acid catalysis is required for protonation and dehydration, because without an acid present the yield is reduced to just 17 %, (Entry 1). Solid phase Amberlite ICR50 H-form is not a suitable alternative reagent, as this reduces the yield to just 7 %, (Entry 2). Conversely, it was found that, as reported in the chemical literature, *p*-TSA is a very successful alternative reagent,<sup>128</sup> and even when utilised in catalytic amounts it leads to excellent yields of 84 %, (Entries 3-5). However, the best conditions identified from this study were the use of AcOH as the solvent, as operationally this is facile and excellent yields are obtained (Entry 6).

Entry	Solvent	Acid	Yield (%) <sup>a</sup>
1	Toluene	-	17
2	Toluene	Amberlite ICR50 H-form	7
3	Toluene	3eq. <i>p</i> -TSA	86
4	Toluene	1eq. <i>p</i> -TSA	84
5	Toluene	Cat. <i>p</i> -TSA	84
6	-	AcOH as solvent	87

<sup>a</sup> Isolated yield.

**Table 10.3:** The effects of solvent on the Friedländer synthesis of quinolines, using 2-amino benzophenone and cyclohexanone.

The use of AcOH as solvent and acid catalyst is highly advantageous to this reaction in terms of results and operational simplicity. The new procedure was simply to heat 2-aminobenzophenone (1 mmol) and cyclohexanone (2 mmol) in AcOH (2 mL) in a MW tube. The use of this procedure has led to higher yields and shorter reaction times (Table 10.4). This system is now so efficient that the reaction proceeds efficiently, even at room temperature, (89 % after 24 h). The best yields are obtained with just a five or ten minute heating period at 160 °C (Entries 2 and 3), with almost optimal yields obtained. Longer heating periods lead to a decrease in isolated yield (Entry 4)

Entry	Temperature (MW heating, °C)	Time (min)	Yield (%) <sup>a</sup>
1	100	5	79
2	160	5	94
3	160	10	94
4	160	20	87

<sup>a</sup> Isolated yield.

**Table 10.4:** The effects of time and temperature on the Friedländer synthesis of quinolones when AcOH is used as the solvent.

Following the identification of the optimal Friedländer conditions, using AcOH as the solvent and heating in a MW for just 5 minutes at 160 °C, further investigations were undertaken to examine the potential of this application. A larger scale reaction was carried

out using 500 mg of 2-aminobenzophenone (2.5 mmol) in the same volume of AcOH (2 mL), thus more than doubling the concentration of starting materials. A yield of 95 % was obtained. This shows no difference due to scale, thus showing this reaction has a high potential for large scale reactions, where minimal solvent use is desired.

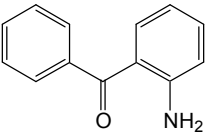
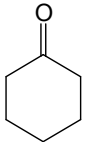
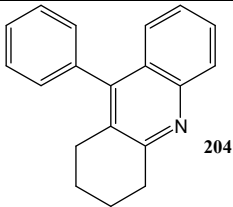
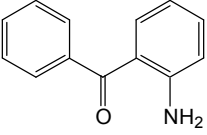
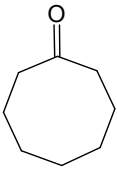
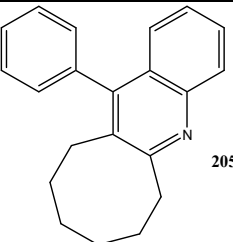
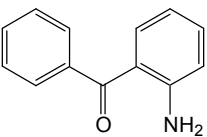
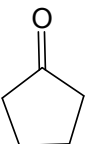
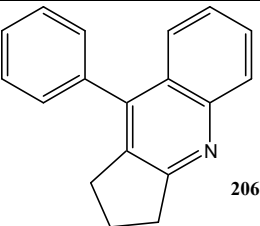
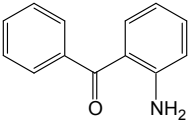
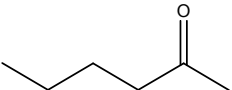
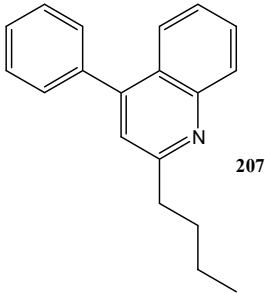
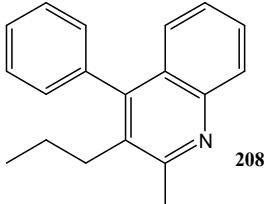
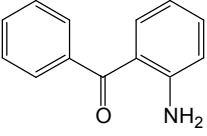
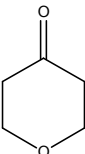
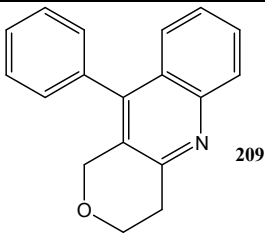
Investigations were also carried out to explore the effect, if any, of reducing the relative amount of ketone used (Table 10.5). There was little difference in yields observed when using 1, 1.5 or 2 equivalents of cyclohexanone. For this reason, the amount of ketone used in all subsequent reactions has been reduced to 1.5 equivalents. An excess was used to ensure complete consumption of the aniline. This ratio could be reduced if required by a particular application.

Entry	Equivalents of cyclohexanone used	Yield (%) <sup>a</sup>
1	2	94
2	1.5	92
3	1	87

<sup>a</sup> Isolated yield.

**Table 10.5:** The use of differing amounts of ketone on the Friedländer synthesis of quinolines, with 2-aminobenzophenone and cyclohexanone.

Now the conditions have been optimised, the new procedure for the Friedländer synthesis of quinolones was used on a range of different starting materials (Table 10.6). Excellent yields were obtained in all cases. The reaction with 2-hexanone showed some degree of regioselectivity as the two possible products were obtained in a 1: 1.9 ratio (Entry 4).

Entry	Starting Material	Ketone	Product	Yield (%) <sup>a</sup>
1			 204	94
2			 205	93
3			 206	99
4			 207	23 <sup>b</sup>
			 208	43 <sup>b</sup>
5			 209	99

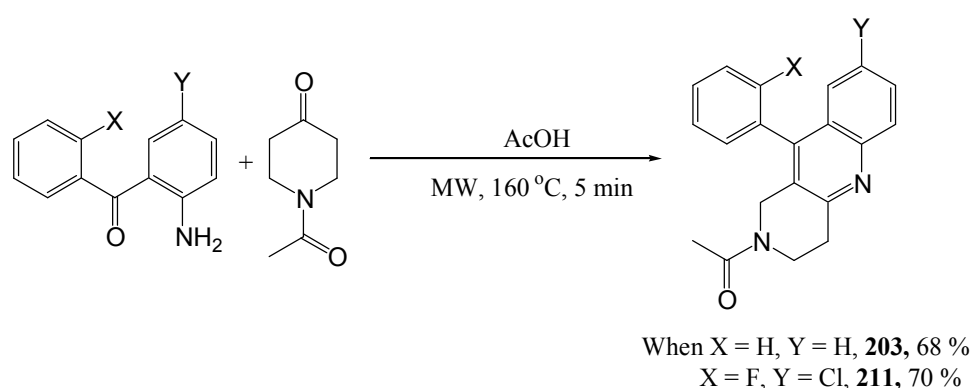
6				91
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<sup>a</sup> Isolated yield.

<sup>b</sup> Regioisomers obtained, 1:1.9, overall yield 66 %.

**Table 10.6:** Microwave assisted Friedländer Synthesis of quinolones.

Application of this new procedure was applied to the original target compounds (Scheme 10.5) affording the expected compounds in excellent yields of 68 and 70 %. However, when 1-acetyl-4-piperidone is used as the ketone, there is an additional problem concerning the removal of the excess starting ketone. The product and the ketone co-elute so cannot be separated by flash chromatography. Although this problem had been reduced by using only 1.5 equivalents of ketone, it was still significant. Luckily, it was easily solved by stirring the crude product with a scavenger resin, PS-TsNHNH<sub>2</sub>,<sup>87</sup> in DCM for 1 hour. The resin is then simply removed by filtration and the final compound purified by flash chromatography. This extra purification may be part of the reason why these yields are lower than with other compounds (Table 10.6) because the overall yield for this step did not actually show any improvement following the optimisation process. The new conditions are, however, faster and more operationally simple.

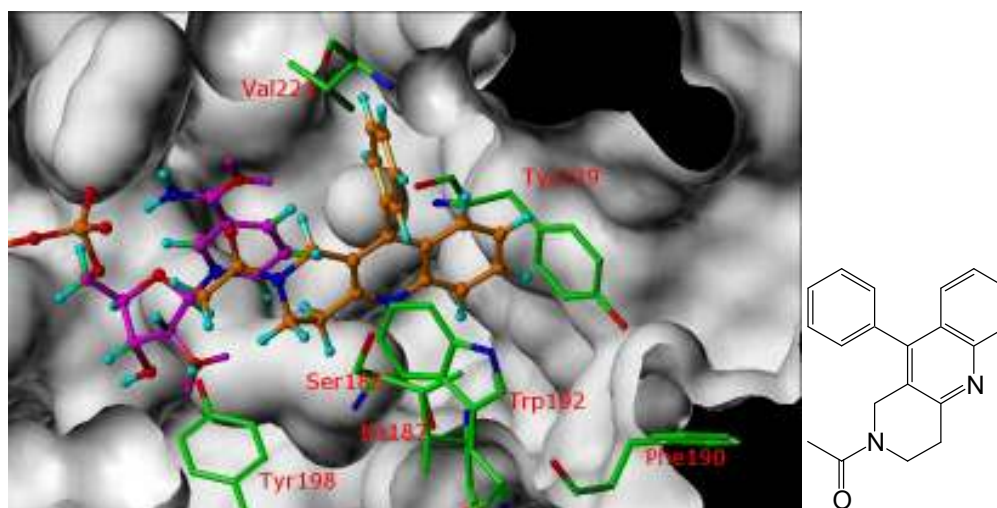


**Scheme 10.5:** Synthesis of quinoline based potential inhibitors, **203** and **211**.

Although compounds **203** and **211** were not the original targets, they do display certain aspects of the original pharmacophore required for activity discussed in Chapter 5. There is

a large hydrophobic head group, with a linking unit attached to a hydrogen bond acceptor. This suggests that these compounds may exhibit some activity. There is, of course, no guarantee that these compounds will have any activity as, although they do have similar regions to the designed compounds, the tricyclic system means that the compounds are more planar and are consequently occupy a very different spatial geometry than the designed target compounds. In order to answer this hypothesis compounds **203** and **211** were submitted for biological evaluation, but no results have yet been obtained. Compounds **203** and **211** have also been submitted for screening in the NCI cancer cell lines (for more details see Chapter 11) these results are also awaited.

Figure 10.4 shows compound **203** docked into the 17 $\beta$ -HSD Type 3 homology model. From this docking some potentially important docking interactions were identified. There is a potential hydrogen bond formed between the aromatic nitrogen of the compound and a Ser185 side chain.

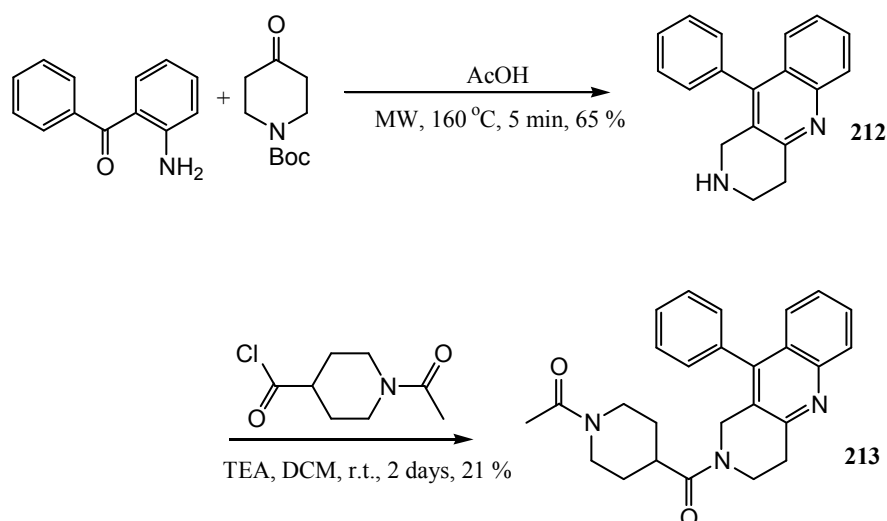


**Figure 10.4:** Compound **203** docked into the 17 $\beta$ -HSD Type 3 homology model.

### 10.3 Synthesis of Extended Analogues

As with the other series mentioned in Chapters 6 and 7, it was likely that these quinoline based compounds (**203** and **211**) would be too short for optimal inhibitory activity, so extended analogues were synthesised (Scheme 10.6). The initial step between 2-aminobenzophenone and 1-Boc-4-piperidone did not proceed as expected. The reaction, did not produce the *t*-Boc protected intermediate. The reaction led to the deprotected free amine intermediate. Although this was not as planned, it means that the synthetic route is actually one step shorter, as the Friedländer synthesis and deprotection occur in one step, in

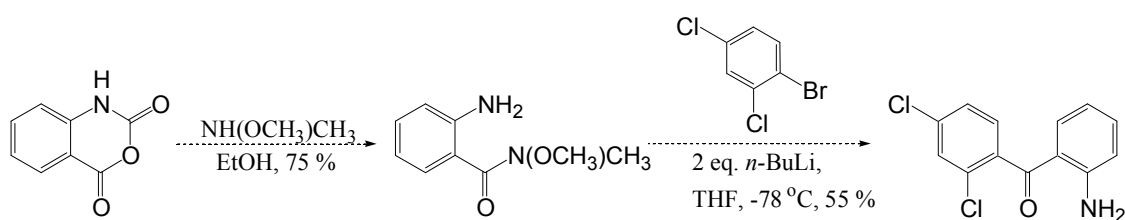
a good yield of 65 %. This does, however, show that acid labile groups are not tolerated by this optimized Friedländer synthesis. The free amine was then reacted with 1-acetyl-piperidine-4-carbonyl chloride using the standard conditions. The final product (**213**) was isolated in 21 % yield. This compound has been submitted for biological evaluation, but the results have not been obtained yet.



**Scheme 10.6:** Synthesis of an extended quinoline based potential inhibitor, **213**.

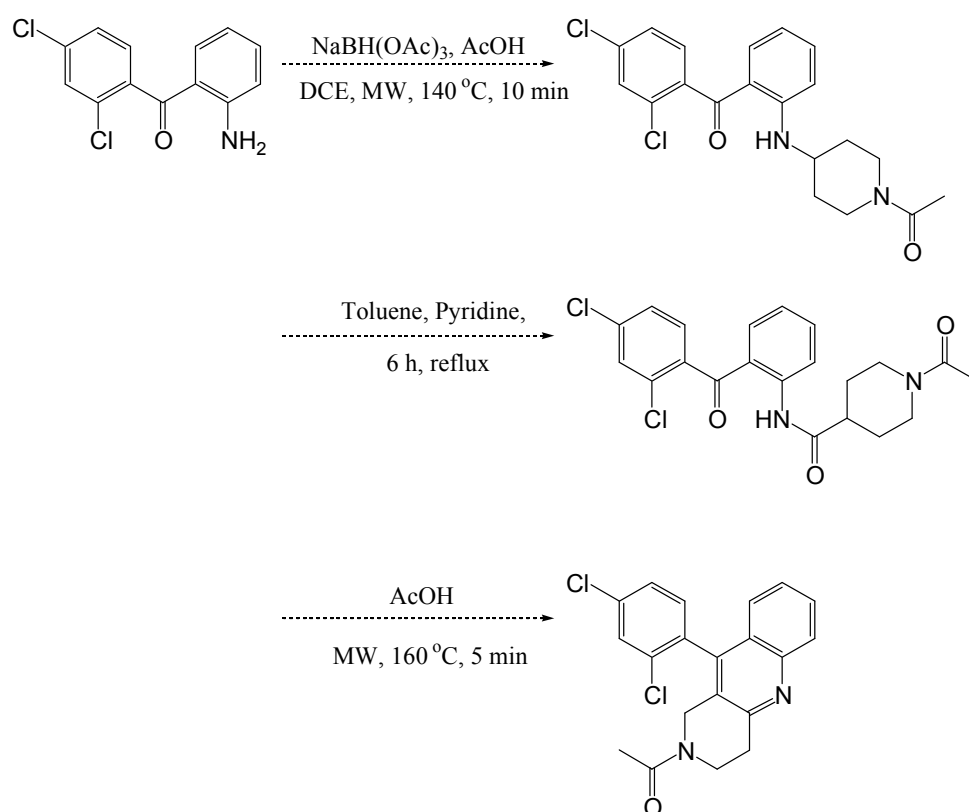
#### 10.4 Future Work in these Series

There is one potential drawback to this series of compounds: the removal of the substitution on the phenyl rings of the hydrophobic headgroup, when compared to all other series mentioned in previous chapters. It is possible that the halogen, trifluoromethyl or trifluoromethoxy substitutions may affect the activity of the compounds, either detrimentally or beneficially. When the biological results are obtained these two series of compounds, the benzophenone linked headgroup and the quinoline based compounds will be assessed to determine whether further investigations are needed. If some activity is shown, then the synthesis of direct hydrophobic headgroup analogues (i.e. 4-chloro, 2,4-dichloro etc.) will be required. Although the desired substitution patterns are not commercially available as 2-aminobenzophenones, they can be easily synthesised from isatoic anhydride (Scheme 10.7).<sup>129</sup> Fyre et al report that 2-amino-4'-chloro-benzophenone can be synthesised using this method, with the second step proceeding with a 55 % yield (Scheme 10.7).



**Scheme 10.7:** Literature Synthesis of substituted benzophenones.<sup>129</sup>

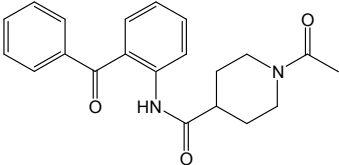
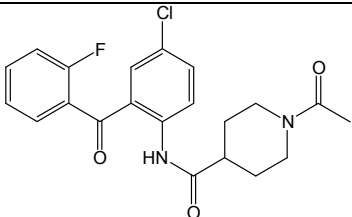
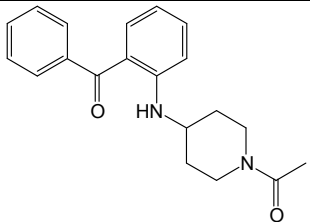
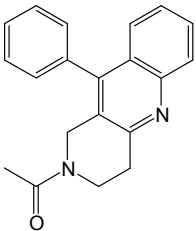
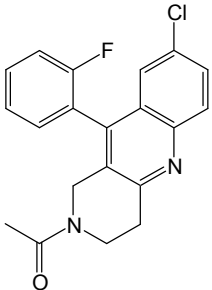
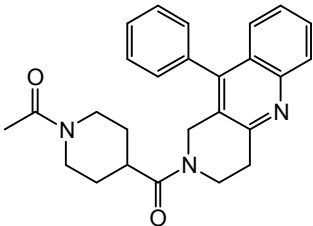
This substituted 2-aminobenzophenone could then be subjected to the synthetic procedures established in this Chapter to produce the substituted analogues of compounds previously synthesised (Scheme 10.8).



**Scheme 10.8:** Future synthesis of potential inhibitors with a substituted hydrophobic headgroup.



## 10.5 Table of Compounds Synthesised within Chapter 10

Compound no.	Structure	% Inhibition (at 10 $\mu$ M). <sup>a</sup>
200		b
201		b
202		b
203		b
211		b
213		

<sup>a</sup> Results obtained from the TLC assay. Mean of at least 2 measurements with typically a SD of  $\pm 5$  %, <sup>b</sup> Not determined.

## Chapter 11

### Results of Other Biological Tests

#### 11.1 Selectivity

It is anticipated that any drug that is selective for the desired target will have lowered toxicity or side effects compared to a less specific compound. It is essential that any 17 $\beta$ -HSD Type 3 inhibitor is selective over the other 17 $\beta$ -HSD isozymes, particularly Type 1 and 2. 17 $\beta$ -HSD Type 1 is involved in the biosynthesis of oestrogens and 17 $\beta$ -HSD Type 2 catalyses the inactivation of active androgens and oestrogens. Inhibition of 17 $\beta$ -HSD Type 2 may lead to the accumulation of active steroids which may cause toxicity problems and other side effects. For this reason the 17 $\beta$ -HSD Type 3 inhibitors were assessed for their inhibitory activity on 17 $\beta$ -HSD Types 1 and 2 in order to establish selectivity.

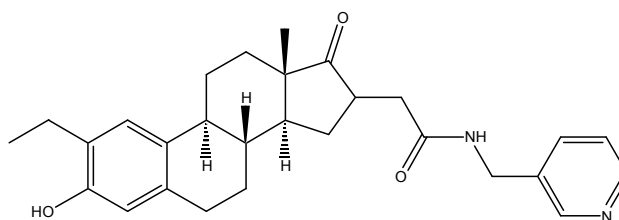
##### 11.1.1 Selectivity over 17 $\beta$ -HSD Type 1

Table 11.1 shows the inhibitory activity data obtained when the active 17 $\beta$ -HSD Type 3 inhibitors were tested in the 17 $\beta$ -HSD Type 1 assay. The assay is of a similar format to the 17 $\beta$ -HSD Type 3 TLC assay. T-47D breast cancer cells, which express a high ratio of 17 $\beta$ -HSD Type 1 to 17 $\beta$ -HSD Type 2, were used to test the conversion of labelled oestrone to oestradiol in the presence and absence of the potential inhibitors. The cells were incubated with <sup>3</sup>H-oestrone at a concentration of 2 nM, in the absence or presence of the inhibitors (10  $\mu$ M). After incubation of the culture for 3 h at 37 °C, the products were isolated and separated by TLC using DCM/ EtOAc (4:1). The amount of oestradiol formed was measured and compared to the control cells where no inhibitor was present.<sup>130</sup> Each assay was carried out in the presence of a positive control (**214**,<sup>131</sup> see Figure 11.1), to ensure reproducible results were being obtained. A selection of those compounds which exhibited significant 17 $\beta$ -HSD Type 3 inhibition were submitted for testing. The results show that the compounds tested are essentially inactive against 17 $\beta$ -HSD Type 1. This is an excellent result and therefore does not preclude these compounds and related series from further investigations.

Compound no.	Inhibition of 17 $\beta$ -HSD Type 3 (IC <sub>50</sub> , nM)	Inhibition of 17 $\beta$ -HSD Type 1 (% , at 1 $\mu$ M <sup>a</sup> or 10 $\mu$ M <sup>b</sup> )	Inhibition of 17 $\beta$ -HSD Type 2 (% , at 10 $\mu$ M)
<b>36</b> (Positive Control)	5	19.6 <sup>b</sup>	18.4
<b>101</b>	700	12.7 <sup>a</sup>	10.9
<b>123</b>	200	nd	20.3
<b>126</b>	900	1.3 <sup>a</sup>	15.9
<b>165</b>	3900	-2.3 <sup>b</sup>	-

<sup>a</sup> Tested at 1  $\mu$ M, <sup>b</sup> Tested at 10  $\mu$ M.

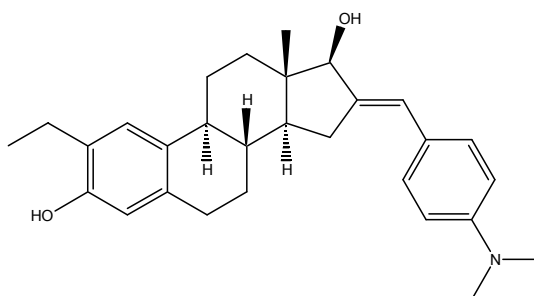
**Table 11.1:** Selectivity of 17 $\beta$ -HSD Type 3 inhibitors of 17 $\beta$ -HSD Type 1 and Type 2.



**Figure 11.1:** Compound **214**, the 17 $\beta$ -HSD1 positive control, (IC<sub>50</sub> = 27 nM).<sup>131</sup>

### 11.1.2 Selectivity over 17 $\beta$ -HSD Type 2

The activity of a selection of 17 $\beta$ -HSD Type 3 inhibitors on 17 $\beta$ -HSD Type 2 was assessed. The results are shown in Table 11.1. The 17 $\beta$ -HSD Type 2 assay uses the same TLC format as the 17 $\beta$ -HSD Type 3 assay. In this case, the assay analyses the conversion of oestradiol to oestrone. MDA-MB-231 human breast cancer cells were incubated with <sup>3</sup>H-oestradiol at a concentration of 2 nM, in the absence or presence of the inhibitor (10  $\mu$ M). After incubation of the culture for 3 h at 37 °C, the products were isolated and separated by TLC using DCM/ EtOAc (4:1). The amount of oestrone formed was measured and compared to the control cells where no inhibitor was present.<sup>130</sup> Compound **215** (Figure 11.2) is used as a positive control in this assay to ensure reproducible results are being obtained.<sup>132</sup> The results in Table 11.1 show that the compounds tested are only weakly active against 17 $\beta$ -HSD Type 2. This is an excellent result and therefore does not preclude the compounds and related series from further investigations.



**Figure 11.2:** Compound **215**, the 17 $\beta$ -HSD2 positive control, ( $IC_{50}$  = 5.4  $\mu$ M).<sup>132</sup>

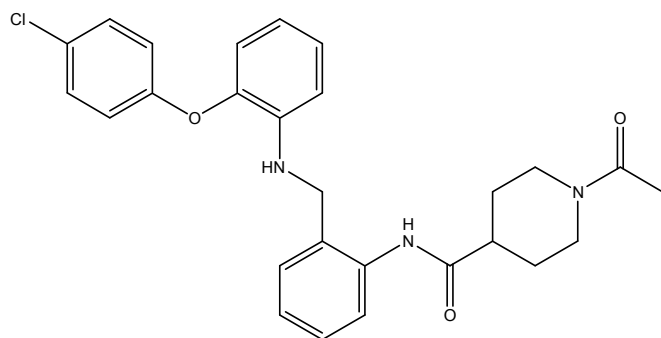
## 11.2 National Cancer Institute Screening Programme

The drug discovery and development arm of the National Cancer Institute (NCI), the Developmental Therapeutics Program (DTP) plans, conducts and facilitates the development of therapeutic agents for cancer.<sup>133</sup> An important part of this programme is the *in vitro* and *in vivo* anti-cancer compound screening program, which aims to identify and evaluate novel chemical leads and biological mechanisms of action. Once compounds have been accepted by the NCI programme, they are subjected to the In Vitro Cell Line Screening Project (IVCLSP). This project screens up to 3,000 compounds a year for any potential anticancer activity. It utilizes 60 different human tumour cell lines, representing leukaemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney. The screening begins with the evaluation of all compounds against the 60 cell lines at a single dose of 10  $\mu$ M. Compounds which exhibit growth inhibition are evaluated against the 60 cell line panel at five concentration levels. Other available testing includes the *in vivo* hollow fibre assay and relevant human tumour xenograft and rodent tumour models.

Selected compounds were submitted to the NCI for screening. As previously mentioned in Chapter 9, compound **196** was analysed, but showed no significant activities in any cell line. The other compounds accepted for testing were:

- Compound **100** exhibited an average  $GI_{50}$  of 15  $\mu$ M. The compound did not show any significant activity on any of the 60 cell lines.
- Compound **126** also exhibited an average  $GI_{50}$  of 15  $\mu$ M. The compound did not show any significant activity on any of the 60 cell lines.
- Compound **165** (Figure 11.3) exhibited an average  $GI_{50}$  of 18.6  $\mu$ M. However, this compound showed a much higher activity in one leukaemia cell line, CCRF-CEM. This is a T lymphoblastoid line obtained from the peripheral blood of a 4 year old Caucasian female with acute lymphoblastoid leukaemia.<sup>134</sup> Compound **172**

exhibited a GI<sub>50</sub> of 10 nM, a Total Growth Inhibition (TGI) of 235 nM and an LC<sub>50</sub> of 5  $\mu$ M. The full data is shown in Figure 11.4. These results are worthy of further investigation, this is in progress.



**Figure 11.3:** Compound **165**, tested for activity in the NCI screen.

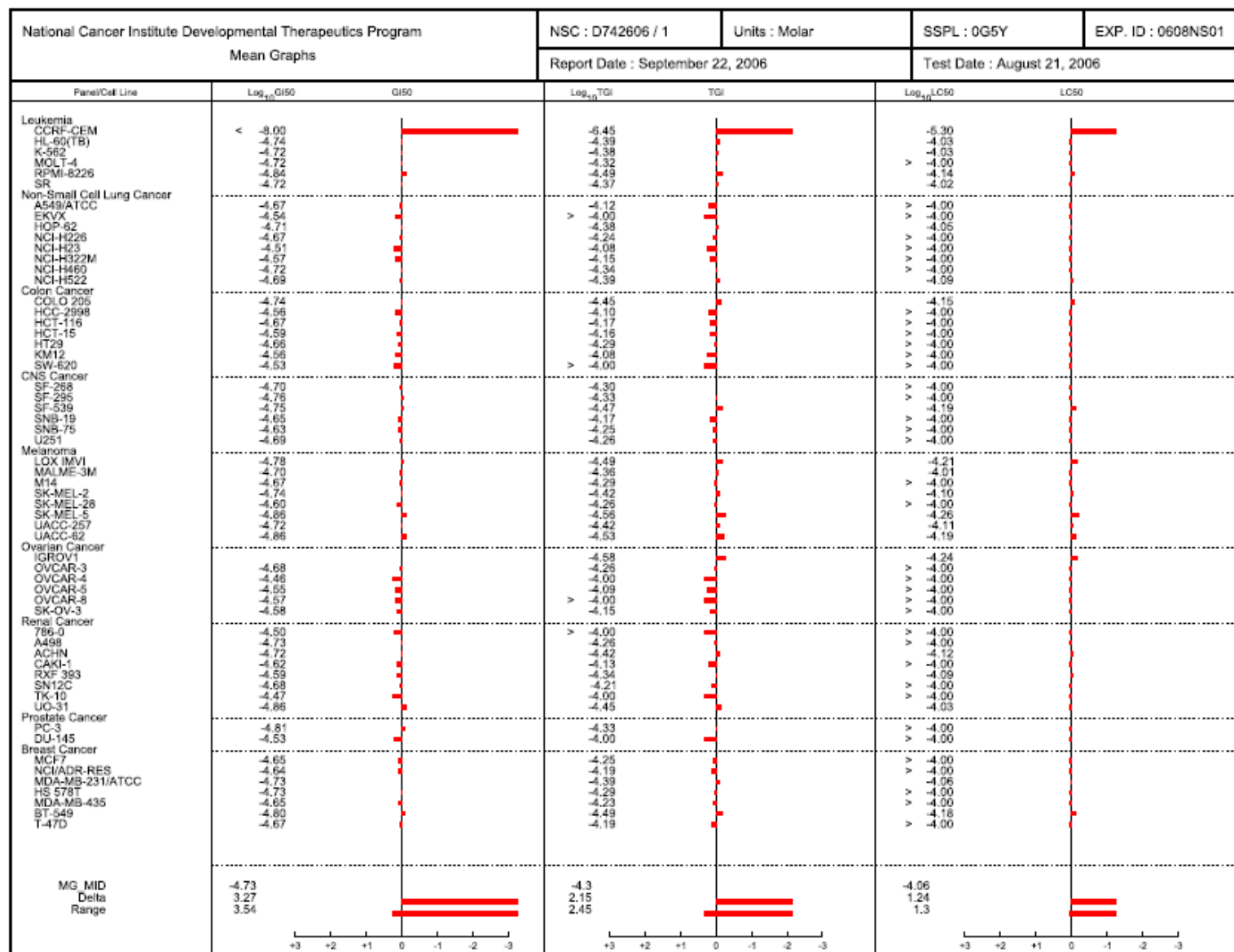
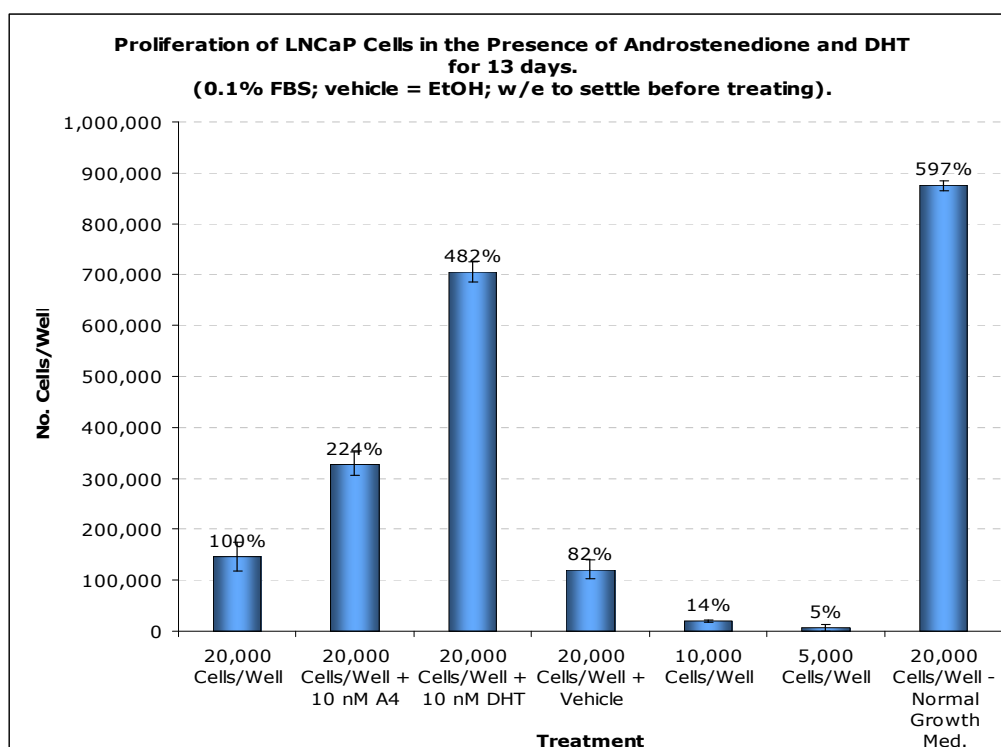


Figure 11.4: Results received from the NCI for compound 165

### 11.3 LNCaP Model used for Efficacy Evaluation

An *in vitro* model was developed by colleagues at St Mary's Hospital (Imperial College, London) using an LNCaP cell, an androgen receptor positive prostate cancer cell line. It can be seen from Figure 11.5 that the growth of the wild type LNCaP cells could be stimulated (~ 4 fold) with DHT, but was only mildly stimulated (~ 2 fold) in the presence of androstenedione (A4).



**Figure 11.5:** Stimulation of wild type LNCaP cells by androstenedione (A4) and DHT.

The stimulatory effects of the androgens was studied in a cell line derived from LNCaPs, LNCaP[HSD3], which had been transfected to have stable expression of 17 $\beta$ -HSD Type 3. These cells were equally stimulated by androstenedione, T or DHT, due to the increased 17 $\beta$ -HSD Type 3 activity (Table 11.2).

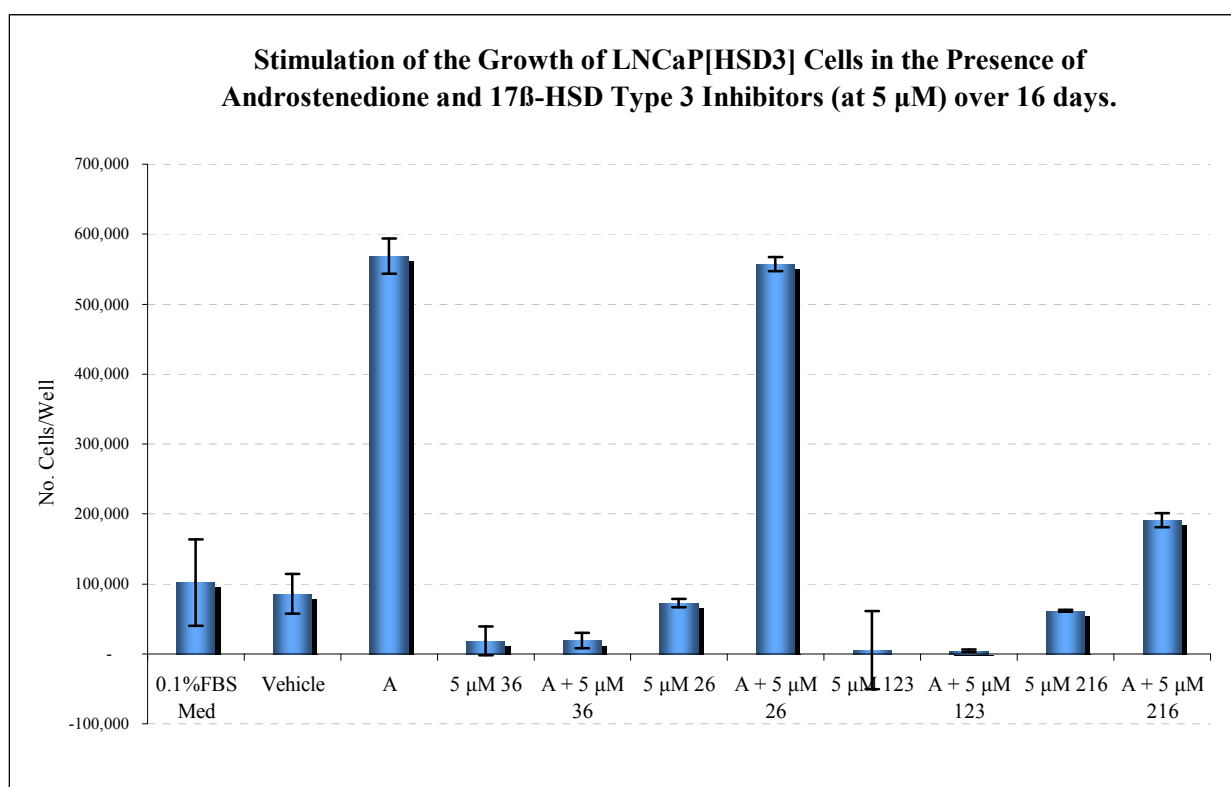
Treatment	Growth
0.1 % FBS	100 % (Control)
10 nM Androstenedione	300 %
10 nM Testosterone	309 %
10 nM DHT	312 %

**Table 11.2:** Androgenic stimulation of LNCaP[HSD3] cell.

This assay was then used to evaluate the *in-vitro* efficacy of some of the most potent compounds in this project so far (Figure 11.6). Those compounds tested were:

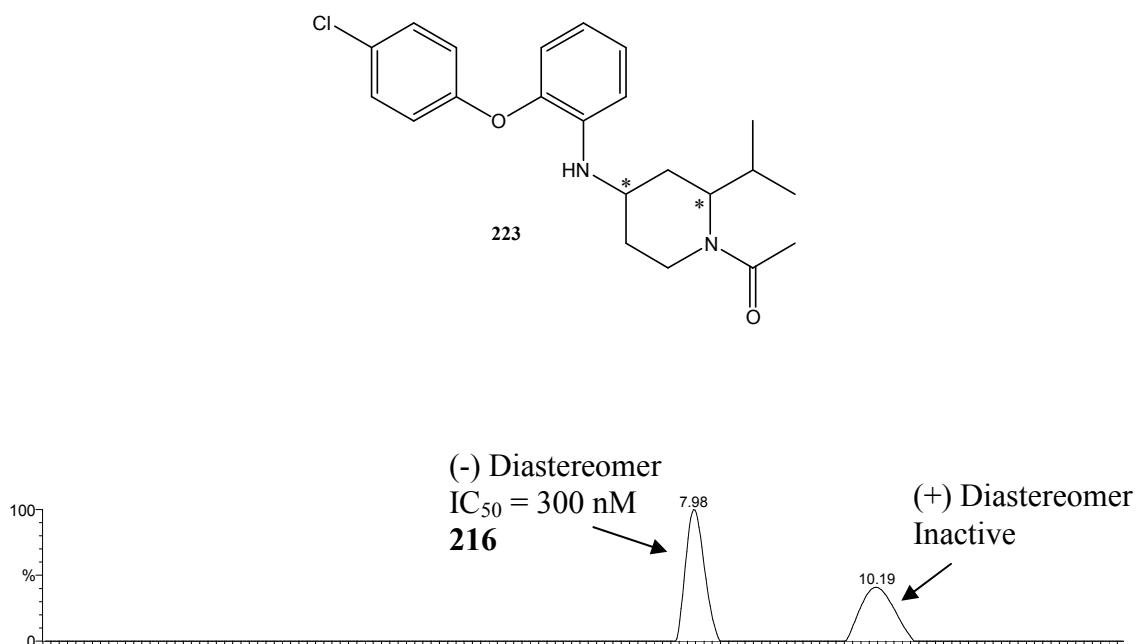
- Compound **36**, the Schering-Plough positive control,  $IC_{50} = 1-5 \text{ nM}$ ,<sup>47</sup>
- Compound **26**, the BMS positive control,  $IC_{50} = 200 \text{ nM}$ ,<sup>45</sup>
- Compound **123**, a potent in-house compound,  $IC_{50} = 300 \text{ nM}$ ,
- Compound **216**, see Figure 11.6,  $IC_{50} = 300 \text{ nM}$ .

Initially, the compounds were assessed at  $5 \mu\text{M}$ . At this concentration, compounds **36** and **123** surprisingly led to substantial toxic effects. In contrast compound **26** was not as toxic, but it did not show any efficacy in this model. Compound **216** (Figure 11.7) a novel in-house inhibitor, showed low toxicity and good efficacy in this model. When the cells were incubated with  $10 \text{ nM}$  of androstenedione and  $5 \mu\text{M}$  of compound **216**, the inhibitor effectively reduced the androstenedione-stimulated proliferation of LNCaP cells, to just 34 % compared to the control cells.



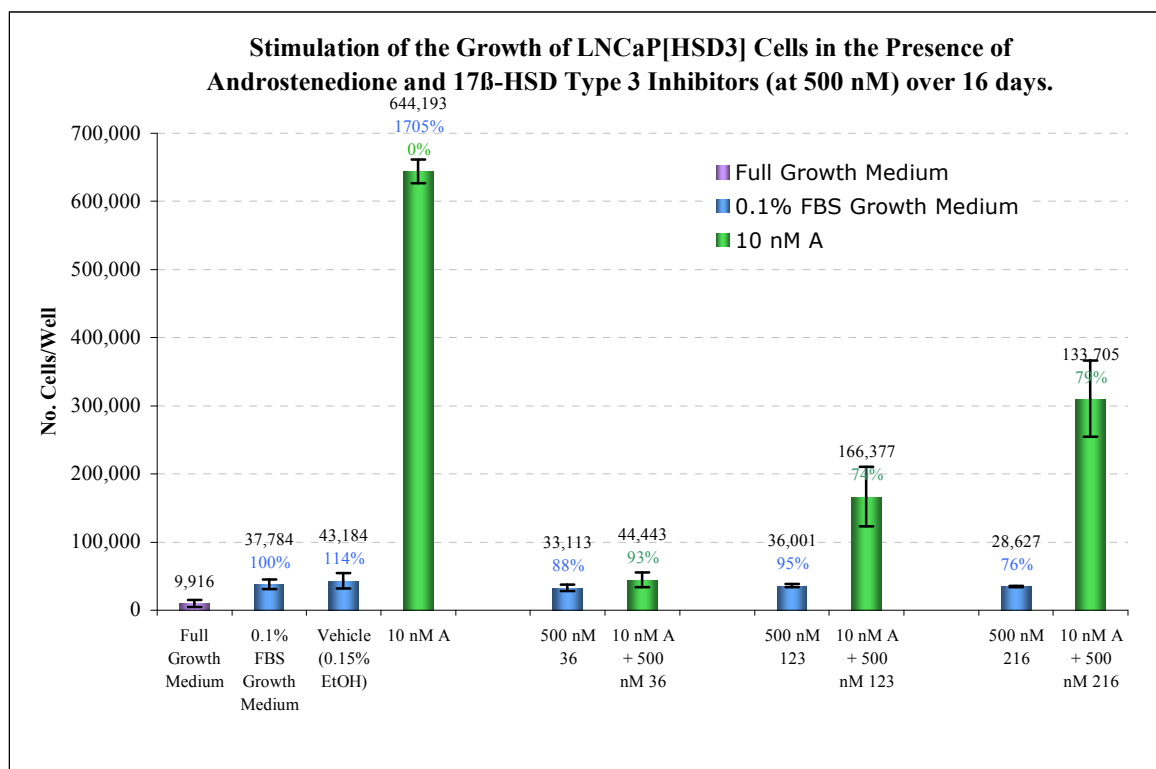
**Figure 11.6:** Growth stimulation by androstenedione (A at  $10 \text{ nM}$ ) and the effects of 17 $\beta$ -HSD Type 3 inhibitors (at  $5 \mu\text{M}$ ) upon this growth.





**Figure 11.7:** Compound **216** and the ChiralPak HPLC trace obtained during the separation of the diastereomers.

The experiment was repeated using the inhibitors at a concentration of 500 nM. At this concentration, compounds **36** and **123** no longer showed the toxicity previously observed at the higher concentration. Therefore, as expected compound **36** showed excellent inhibitory activity of 93 %. Compound **123** also showed excellent activity, causing 74 % inhibition of stimulation. When compound **216** was evaluated at this lower concentration it exhibited a percentage inhibition of 52 %. This shows that currently compound **123** is the most efficacious compound synthesised and tested in-house so far.



**Figure 11.8:** Growth stimulation by androstenedione (A at 10 nM) and the effects of 17 $\beta$ -HSD Type 3 inhibitors (at 500 nM) upon this growth.

The results of these preliminary studies showed that the competitor compound **26** has significant problems with efficacy. Therefore, this compound is not suitable for use in the development of a 17 $\beta$ -HSD Type 3 *in vivo* assay. However, compounds **36**, **123** and **216** (Figure 11.7) show good potency and good efficacy in this model. This means that these compounds are promising candidates for the development of a 17 $\beta$ -HSD Type 3 *in vivo* assay and investigations are on-going.

## Chapter 12:

### Summary, Structure Activity Relationships and Conclusions

#### 12.1 Introduction

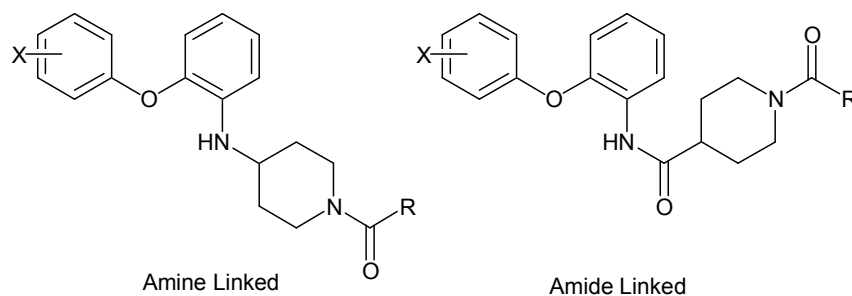
Prostate cancer represents a massive unmet medical need and remains a great challenge to doctors, patients and researchers. It is known that prostate cancer growth is often stimulated by the presence of androgens and that growth of androgen dependent prostate cancer can be reduced with androgen depletion. The enzyme 17 $\beta$ -HSD Type 3 is involved in the biosynthesis of androgens. It catalyses the synthesis of androstenedione into testosterone, which is in turn converted to DHT, the most active androgen. Inhibition of 17 $\beta$ -HSD Type 3 would lead to a lower level of testosterone and therefore DHT. It is anticipated that a reduction in androgen levels would reduce the growth of prostate cancer. Proof of concept has been demonstrated by Schering-Plough with their potent inhibitors. This was discussed in detail in Chapter 1.<sup>51, 52</sup> The compounds reported by Schering-Plough are just some of the 17 $\beta$ -HSD Type 3 inhibitors reported in the scientific literature. These compounds show excellent potency but, as yet, none is clinically significant.

One of these potent compounds, **36** was chosen as a positive control compound for use in development of the in-house biological assays. However, due to the exceptional potency of this compound in comparison to the current in-house synthesised compounds, problems with sensitivity were experienced. An alternative compound **26** was then selected as a new positive control. This compound has been successful as a positive control and is now used as such in all assay development and testing.

During the course of this project the biggest challenge has been the development of a successful and efficient biological assay. The original TLC assay was undertaken using a transfected 293-EBNA cell line with stable human 17 $\beta$ -HSD Type 3 expression. This was a successful assay which gave reliable results. However, it was desired to change the format of the assay to a high-throughput assay, using the DELFIA format. This was highly problematic and it has been decided that attention will revert back to using the TLC assay, until a later stage in the project where the DELFIA assay may be more suitable for use with hopefully more potent compounds. These problems have unfortunately led to a significant delay in obtaining biological results in the project. This means that much of the biological activity information has not been obtained so far and thus a lot of potentially useful SAR information from this thesis is unavailable. These results will be obtained at a later date.

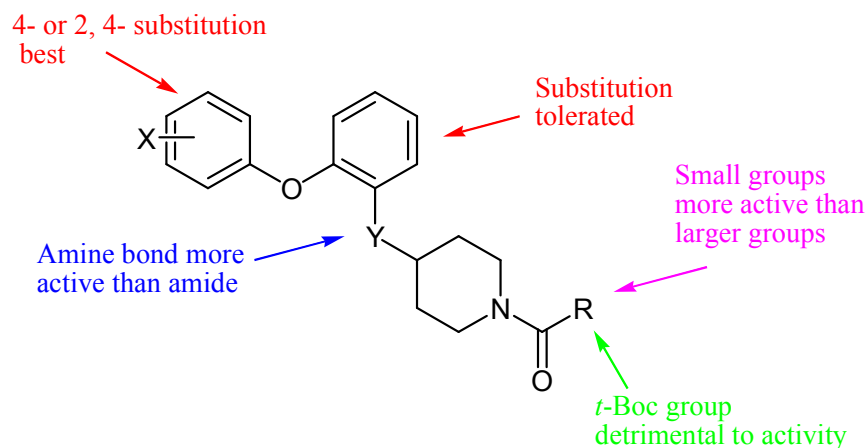
## 12.2 Amide and amine linked targets

The initial targets in this project were established by the use of pharmacophore modelling which identified 4 main regions that were likely to be important to activity. This was discussed in more detail in Chapter 5. A series of compounds based around a diphenylether hydrophobic headgroup and a piperidine ring was designed (Figure 12.1).



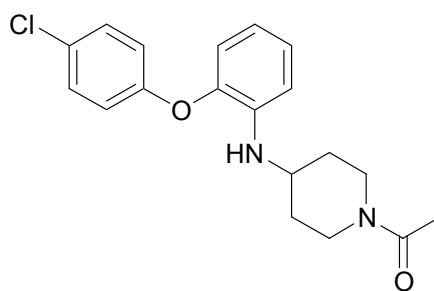
**Figure 12.1:** Amine and amide linked initial targets.

A range of compounds in both series were synthesised (see Chapters 6 and 7) and the biological data for some compounds have been obtained. From the data currently available some SAR information for these series can nevertheless be established. These are shown in Figure 12.2.



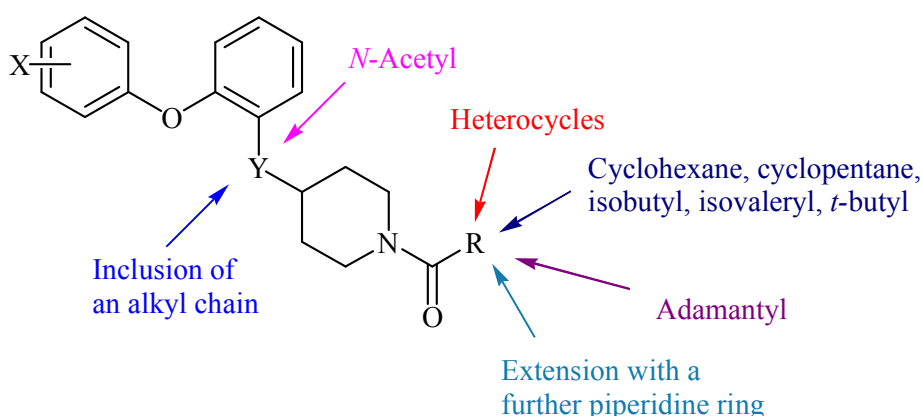
**Figure 12.2:** SAR diagram of the original amide and amine linked target compounds.

The first potent compounds of the project were synthesised in the amine linked series. The most potent compound was **101** (Figure 12.3). This compound has an IC<sub>50</sub> of 700 nM and has been subjected to selectivity testing for 17 $\beta$ -HSD Types 1 and 2. No significant activity was observed in either assay, thus showing **101** to be an active, selective 17 $\beta$ -HSD Type 3 inhibitor.



**Figure 12.3:** Compound **101**, the most potent compound in the amine or amide linked series.

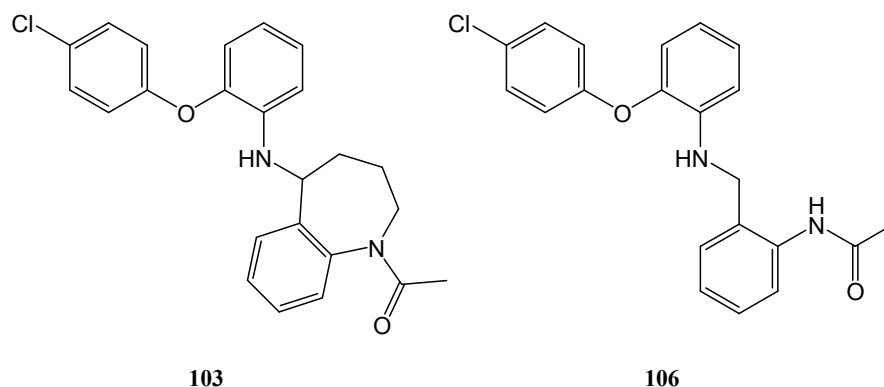
There is much biological information about these series of compounds still to be obtained. Figure 12.4 shows some of the SAR information that should be established once full biological results have been obtained. This includes the effects upon activity when the compounds are extended with a further piperidine ring. The docking studies of these compounds have indicated that they should show enhanced activity over the shorter, original targets.



**Figure 12.4:** Areas of the original amide and amine linked target compounds where biological activity information is still to be established from the compounds awaiting biological testing.

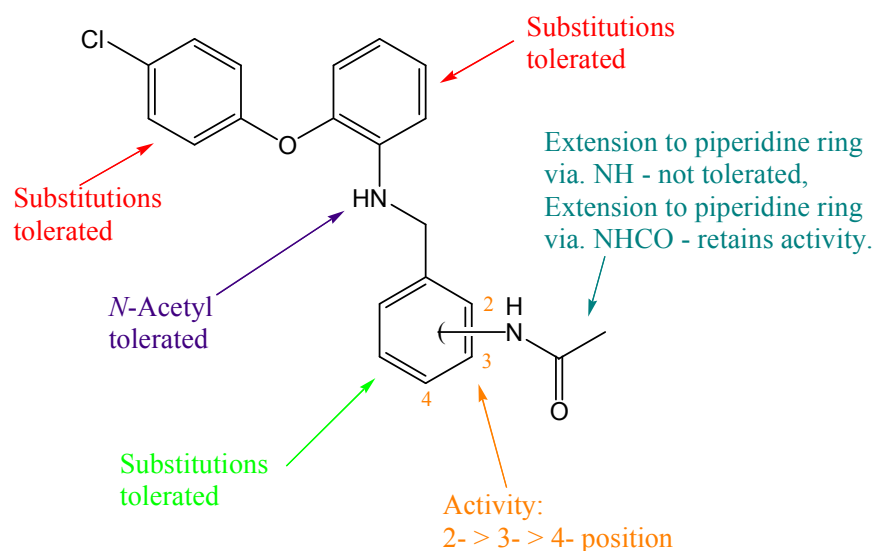
### 12.3 Benzylamine Linked Series

Throughout the course of the project a 17 $\beta$ -HSD Type 3 homology model was used to aid structure based drug design. The docking studies using compounds from the amide and amine linked series showed an area of space around the central piperidine region. Attempts to utilise this space have been successful, including compound **123** (Figure 12.5) and the benzylamine linked series of compounds (Chapter 8). The initial target, compound **126** was synthesised and showed excellent inhibitory activity, with an IC<sub>50</sub> of 900 nM.



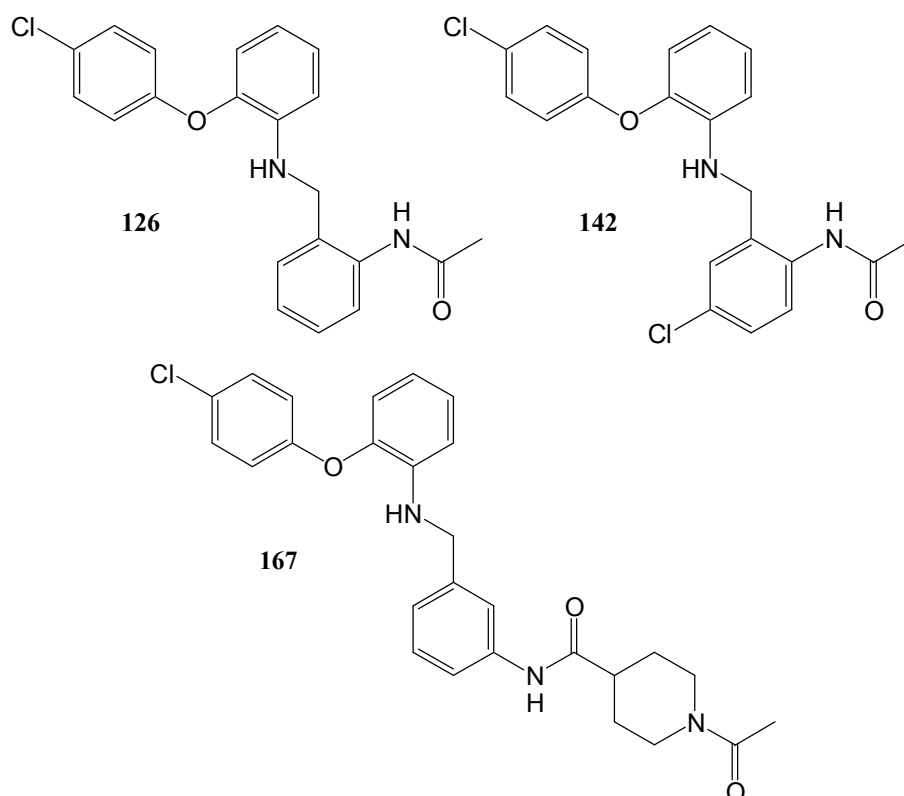
**Figure 12.5:** Compound **123** ( $IC_{50} = 300$  nM) and **126** ( $IC_{50} = 900$  nM).

Extensive modifications of this template were then undertaken. However, in this series, only limited biological data has been obtained. From the data obtained some SAR conclusions can be drawn, these are shown in Figure 12.6.



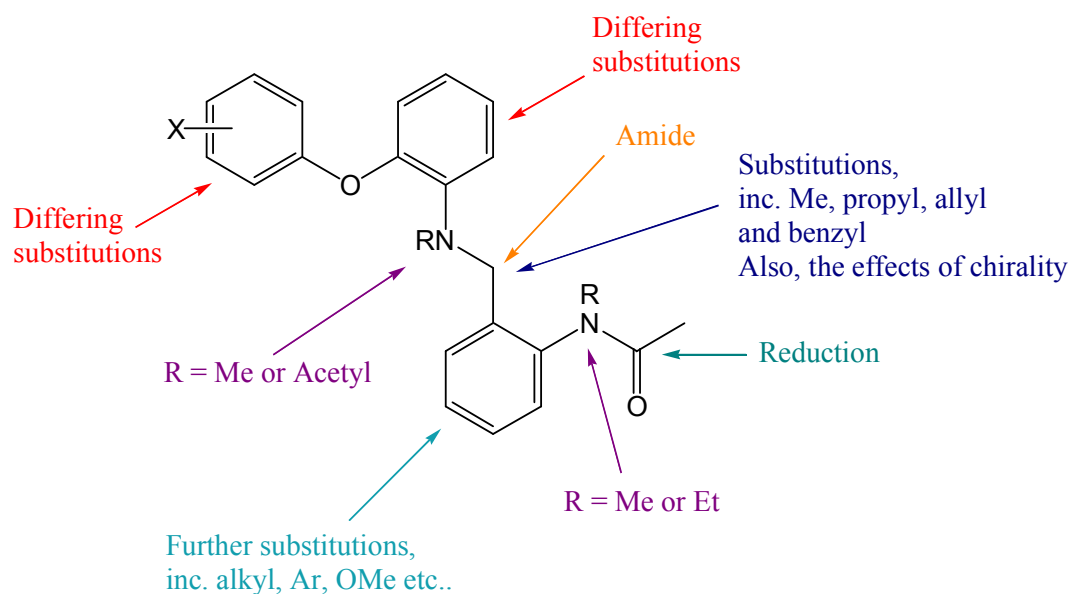
**Figure 12.6:** SAR diagram of the benzylamine linked targets.

The best compounds tested so far are the original target **126** ( $IC_{50} = 0.9$   $\mu$ M) and compounds **142** ( $IC_{50} = 1.9$   $\mu$ M) and **167** ( $IC_{50} = 1.1$   $\mu$ M). The structures are shown in Figure 12.7. Compound **126** has been tested for selectivity against 17 $\beta$ -HSD Types 1 and 2. No significant activity was observed, thus showing **126** to be a potent, selective 17 $\beta$ -HSD Type 3 inhibitor.



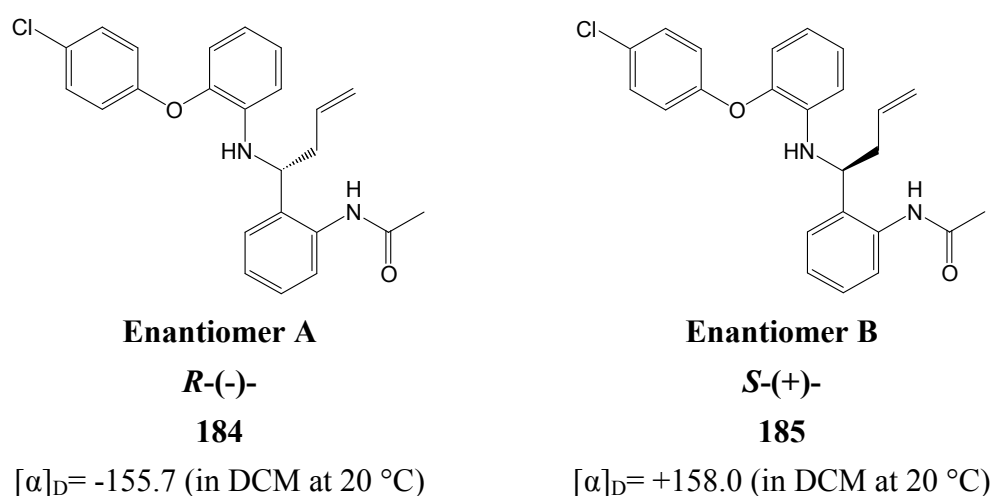
**Figure 12.7:** The most active inhibitors in the benzylamine series.

There are many compounds synthesised in this series (>30), most of which have yet to undergo biological testing. Once obtained, this information will help to establish a robust SAR for this series. Figure 12.8 shows some of the SAR information that should be established once full biological results have been obtained.



**Figure 12.8:** Areas of the benzylamine based target compounds where biological activity information is still to be established from the compounds awaiting biological evaluation.

An important area of investigation in this series was the effects of chirality on the biological activity, as the introduction of substitutions onto the CH<sub>2</sub> of compound **126** leads to the formation of a chiral centre. The potential importance of this was investigated using racemic compound **172** as an example. The two enantiomers (**184** and **185**) were separated by chiral HPLC and X-ray crystallography was used to determine the absolute configuration. The optical rotations were also measured and found to be directly opposing for the two enantiomers. This confirmed enantiomer A (**184**) to be the *R*-(-)- enantiomer and compound **185** to be the *S*-(+)- enantiomer (Figure 12.9).



**Figure 12.9:** Enantiomers from racemic compound **172**.

The two separate enantiomers and the racemic mixture are now awaiting biological evaluation. It is hoped that the results received will establish whether inhibitory activity is affected by the chirality of the compound within this series and therefore will give vital information regarding the size and shape of the enzyme active site.

Within this benzylamine linked series, many alternations to the original template are possible and many compounds have been synthesised to investigate how these modifications affect the inhibitory activity of the compounds. However, none of the modifications has shown an improvement in potency when compared to the original target, **126**. It is hoped that some of the compounds awaiting evaluation will exhibit an improvement in potency. Once all the information has been obtained it is also possible that there may be additive effects and the combination of successful modifications would be a possible way to see an enhancement in potency.



## 12.4 Microwave Assisted Synthesis and Novel Methodology

Throughout the course of this project there has been an emphasis upon investigating novel methodology and technology as the need arose. During the synthesis of the original amide and amine linked compounds (Chapters 6 and 7) it was found that the synthesis of the aromatic amine bond was highly problematic. This was most likely due to the low reactivity of the anilines being used. The introduction of MW assisted synthesis greatly increased the yields and reliability of the reaction, whilst ensuring fast reaction times. This methodology proved itself to be a highly valuable asset, and it can be applied to reactions with a wide range of ketones, aldehydes and anilines.<sup>85</sup> This reaction has proved to be highly useful and was used extensively within the project. When a reaction between an aniline and an aldehyde was desired, it was found that the reaction proceeded effectively at room temperature, the use of MW technology was no longer required. This methodology, either using the MW or at room temperature led to the synthesis of all the amine linked compounds and the majority of the benzylamine linked compounds.

This project also led to the development of an operationally simple, efficient, fast and reproducible modification of the Friedländer synthesis of quinolines. The new MW methodology allows the reaction between 2-aminophenylketones and cyclic ketones to proceed to form the desired quinoline scaffold in just 5 minutes in almost quantitative yields. This has great advantages over the high temperatures or strong acids required in previously reported methods (for more details see Chapter 10).

## 12.5 Conclusions

This project set out to explore the inhibitory potential of compounds against 17 $\beta$ -HSD Type 3, with the aim to develop clinically significant treatments for androgen dependent prostate cancer. Although this aim is likely to be many years away, this project has identified some potentially significant series of compounds, which will require further optimisation. Full biological activity data is still awaited for many of the compounds synthesised but, once obtained, a much clearer picture of the hoped for success of these series will be attained. This will help to construct an SAR, which could then be developed into an effective QSAR to aid *in-silico* predictions of compound activity. This will all help to bring the project closer to the ultimate aim of a safe, effective, clinically successful treatment for prostate cancer and other androgen dependent diseases and disorders.

## Chapter 13

### Biological Experimental Details: Thin Layer Chromatography (TLC) Assay.

#### 17 $\beta$ -Hydroxysteroid Dehydrogenase Type 3 Activity in the Presence or Absence of Regulatory Agents.

(Based upon STERIX S.O.P. No. : 20041007v1.0)

##### 1.0 Purpose

To determine *in vitro* 17 $\beta$ -hydroxysteroid dehydrogenase Type 3 activity in the stable 17 $\beta$ -HSD3-transfected 293-EBNA cell line, 293-EBNA[17 $\beta$ -HSD3], in the presence or absence of inhibitor compounds.

##### 2.0 Assay Procedure for 17 $\beta$ -Hydroxysteroid Dehydrogenase 3.

###### 2.1 Plating of cells

Plate cells at 50,000 / well in 24 well plates in complete growth medium (10 % FBS) without G418 or Hygromycin B 48 hours before assay. Leave 3 wells empty.

###### 2.2 Assay medium

When ready to assay, prepare the assay medium: To 500 mL MEME medium add 5 mL 100 x Pen/Strep, 5mL 100 x L-Glutamine, 5 mL 100 x NEAA and 5 mL 7.5 % sodium bicarbonate solution.

###### 2.3 Substrate medium

Prepare 50 mL 2-3 nM <sup>3</sup>H-Androstenedione substrate medium per 24 well plate to be assayed. Evaporate the volume of <sup>3</sup>H-A required to achieve a final concentration of 2-3 nM to near dryness in a glass test tube before adding to 50 mL assay medium. Vortex to ensure substrate solution is well mixed.

###### 2.4 Inhibitor dilution

Prepare inhibitors at required dilutions each in 6mL substrate solution in 30 mL sterilin tubes. Aliquot 12 mL to use for triplicate Blank and Control wells.

###### 2.5 Cell treatment

Add 1.5 mL substrate medium +/- inhibitor (I) per well to triplicate wells, ensuring all controls are present as follows:

Wells 1-3:	No cells: Blank:	1.5 mL substrate medium / well.
Wells 3-6:	Cells: Control:	1.5 mL substrate medium / well.
Wells 7-21:	Cells: Assay:	1.5 mL substrate medium +/- I / well.
Wells 21-24:	Cells: Count	1.5 mL complete growth medium / well.

## 2.6 Incubation

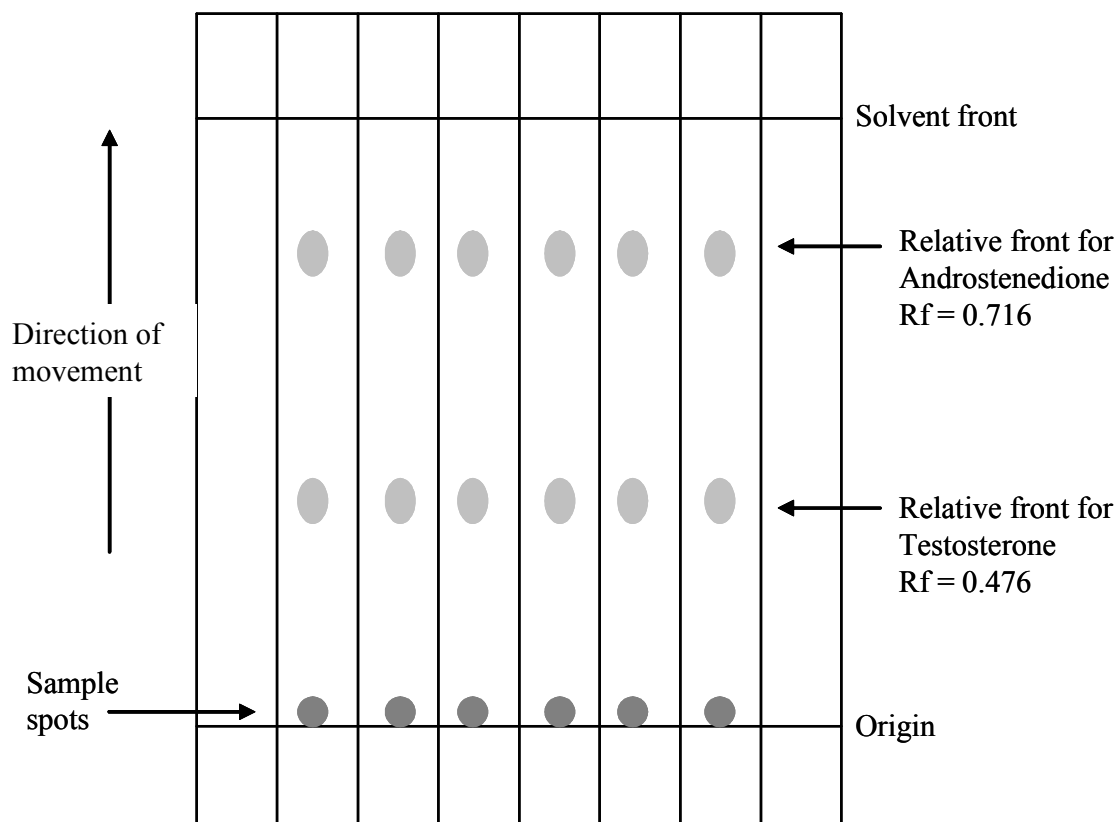
Return to cell culture incubator for 2 hours.

## 2.7 Extraction

After 2 hours take 1 mL medium from each well (except wells 21-24) and place in 125 x16 mm glass test tubes containing 25 µl of recovery solution (containing approx. 5000 cpm  $^{14}\text{C}$ -T and 25 µg unlabelled T). Mix and add 4 mL of diethylether before vortexing on the multi-tube vortexer for 2 x 30 sec, starting on speed setting 4 and gradually increasing to 8. Allow the samples to settle into two phases then freeze the aqueous phase of the sample mixture by placing the tubes in a mixture of dry ice and methanol. When the aqueous phase is frozen (~ 3 min), the upper organic phase should be decanted into labeled 75 x 12 mm tubes and evaporated to dryness under an airstream using the sample concentrator set at 40 °C.

## 2.8 Preparation of TLC plate

While the ether is evaporating, the TLC plate is prepared as in Figure 1.



**Figure 1.** Diagram illustrating the thin layer chromatography plate.

- Origin = 2 cm from the bottom of the plate (marked by pencil).
- Side edge = 1 cm (marked by scoring).
- Each lane = 1.5 cm wide (marked by scoring).
- The lane edges are scored to prevent crossing over of samples between lanes (a mask should be worn to prevent inhalation of silica dust).

## **2.9 Loading the TLC plate**

Remove the evaporated samples from the concentrator after approximately 30 minutes. Add 8 drops of ether to Tube 1 to dissolve the residue, vortex for 20 s, and add the sample drop-wise to the center of the origin in Lane 1 allowing the spot to dry between each drop. Add another 3 drops of ether to Tube 1, vortex for 10 s, and add drop-wise to the spot on Lane 1 of the TLC plate. Repeat the spotting process with each sample on a new lane.

## **2.10 TLC**

When all samples have been loaded onto the plate, leave it to dry completely and prepare the mobile (solvent) phase for the TLC. The mobile phase consists of 4:1 v/v DCM: EtOAc. Therefore mix 80 mL DCM and 20 mL EtOAc gently in a measuring cylinder and pour into a clean TLC tank. Replace and weigh down the lid of the tank.

When the solvent has saturated the tank (around 30 minutes) the TLC plate (or plates) should be carefully placed into the tank so that the base of the plate (but not the origin) is immersed in the solvent. The tank should then be closed and weighted, and the plate left in the tank for about 90 minutes to enable the mobile phase (solvent front) to move to within one or two cm from the top of the plate. Remove the plate and leave to dry flat for approximately 5 minutes before observing the plate under a UV lamp, and locating and marking the major spots (denoting testosterone) in pencil (at high concentrations inhibitor spots may also become visible by UV).

## **2.11 Elution and radioactivity determination**

The spots circled on the TLC plate should be cut out as a square with a gap around the spot to allow for any of the silica that might fall away. These should then be placed in individual scintillation vials containing 0.5 mL methanol. These are then shaken lightly and left for 15 min to elute the radioactivity. The vials are then shaken again before adding 10 mL of EcoScint A (scintillation fluid) to each tube along with 0.5 mL assay medium.

Before the counting the samples, prepare three “total  $^3\text{H}$ ” vials, each containing 0.5 mL Substrate medium, 0.5 mL methanol, and 10 mL of EcoScint A; and three “total  $^{14}\text{C}$ ” vials, containing 25  $\mu\text{L}$  of recovery solution, 0.5 mL Assay medium, 0.5 mL methanol and 10 mL EcoScint A. The “total radioactivity” vials are needed to determine the original level of  $^3\text{H}$ -A and  $^{14}\text{C}$ -T added at the start of the assay in order to make the final activity calculations.

The vials are lidded, labelled, and shaken, and then checked to ensure that all the TLC pieces are lying horizontally on the bottom of the vial so as not to affect the count. The samples are placed in a rack in the scintillation spectrometer and counted using the program for dual [ $^3\text{H}/^{14}\text{C}$ ] isotopes.

## 2.12 Cell count

The medium should be removed from the remaining 3 wells (wells 22-24) and the no. of cells / well counted.

## 3.0 Calculation

To determine the enzyme activity and percentage inhibition.

### 3.1 Corrections to the raw data.

Overall there are 4 corrections that need to be applied to the raw data:

**3.1.1. Crossover correction:** to correct for the percentage crossover of  $^{14}\text{C}$  into the  $^3\text{H}$  channel (usually 12-14 %).

**3.1.2 Recovery correction:** to correct for the proportion of product recovered from the assay (i.e. to account for procedural losses).

**3.1.3 Blank correction:** to correct for any non-enzymatic degradation of substrate to product.

**3.1.4. Dilution correction:** to correct for any dilution that was made prior to eluting the activity.

### 3.2 Data input and calculation.

First, put the raw data into a spreadsheet program (e.g. MS Excel):

- Enter the  $^3\text{H}$  count data in column **A**, and the  $^{14}\text{C}$  count data in column **B**.
- 12% of **B** ( $^{14}\text{C}$ ) should be calculated in column **C** (cross-over).
- Column **D** = **A** - **C** (this corrects the 12 % cross-over factor).
- Column **E** = **B**  $\div$  mean “total  $^{14}\text{C}$ ” (the proportional recovery).
- Column **F** = **D**  $\div$  **E** (this corrects the recovery).
- Column **G** = **F** - mean **Blank** (from column **F**).

- Activity (fmol/2hr/well) = Column **H** = **G x Constant** (see below):
  - **Constant** = (1.5 mL/ 1.0 mL) ÷ (1.5 mL/ 0.5mL x total <sup>3</sup>H cpm) x fmol <sup>3</sup>H-A per well.
  - $\approx (1.5 \div \text{total } ^3\text{H-A cpm in 1.5 mL (e.g.} \sim 150,000)) \times \sim 3000 \approx 0.03 \text{ fmol.}$
  - It accounts for the different volumes used in the sample and the “total <sup>3</sup>H” vial counts, and the specific activity of the <sup>3</sup>H-A.
- Activity (fmol/2hr/million cells) = Column **I** = **H ÷ (mean no. cells per well (millions))**.
- Activity (% of control) = Column **J** = **100 x (Sample I ÷ mean Control I)**.
- Column **K** = sample name
- Column **L** = mean activity of triplicate
- Column **M** = (100 – L) = mean % inhibition of triplicate
- Column **N** = standard deviation of triplicate (%)

#### 4.0 Other notes

The substrate and inhibitors are not stable at RT or 37°C (they must be kept on ice until required). <sup>3</sup>H-Androstenedione and <sup>14</sup>C-testosterone are checked for purity every 6 months, and purified when necessary using the TLC system described.

## Chapter 14

### Biological Experimental Details: DELFIA® Assay.

#### **17 $\beta$ -Hydroxysteroid Dehydrogenase Type 3 Activity in the Presence or Absence of Regulatory Agents.**

##### **1. Maintenance of 293-EBNA cells**

293-EBNA [17 $\beta$ -HSD3] cells are maintained in Biocoat (poly-D-lysine coated) T75 flasks (Becton Dickinson 356537) in 20 mL of Dulbecco's Modified Eagles Medium (DMEM, Sigma D6171) supplemented with 10 % foetal bovine serum (FBS), 1 mM L-glutamine and 250  $\mu$ g/mL G418 and 250  $\mu$ g/mL Hygromycin B in a humidified 5 % CO<sub>2</sub> incubator at 37 °C.

Cells are passaged, twice a week (Tuesdays and Fridays), 1 in 10 split. Assays can be performed three times each week; cells are plated on Friday (for assay Monday), Monday (for assay Thursday) and Tuesday (for assay Friday).

##### **2. 17 $\beta$ HSD3 Assay Methods**

###### **2.1 Plating of cells**

Cells are plated at a density of 15,000 cells per well in 200 $\mu$ l of DMEM supplemented with 10 % FCS and 1mM L-Glutamine in Biocoat (poly-D-lysine coated) 96 well tissue culture plates (Becton Dickinson 356461), 72 hours before use in assays (i.e. Mondays, Tuesdays and Fridays).

Outer wells are filled with 200  $\mu$ l of media and only the inner 60 wells are plated with cells, as there is significant evaporation over the 72 hours prior to the assay. This causes assay drift. Alternatively, it may be possible to seed the cells at a higher density and used 24 hours after plating to reduce the evaporation effect.

###### **2.2 Assay Medium**

Assay medium is prepared immediately prior to the assay: (DMEM supplemented with 1x pen/strep (Invitrogen 15070-063), 1x L-glutamine (Invitrogen 25030-024), 1x NEAA (Invitrogen 11140-035) and 0.075 % sodium bicarbonate (Sigma S-8761).

###### **2.3 Compound Dilution**

Prepare 10 mM stock solutions of the compounds in 100% DMSO. Dilute stock solutions to 100 times the required concentration in DMSO, add 5  $\mu$ l of the DMSO stock solution to 245  $\mu$ l of assay medium.

## **2.4 Androstenedione Dilution**

Prepare a 100nM androstenedione solution in 0.001 % DMSO: assay medium from the 10 mM androstenedione stock in DMSO. Add 25 µl of the substrate to 25 µl of pre-incubated compounds.

## **2.5 Compound and substrate addition**

Remove 200 µl of growth medium

Add 25 µl of compound in assay medium

Incubate for 30 minutes in the humidified CO<sub>2</sub> incubator

Add 25 µl of 100 nM androstenedione

Incubate for 120 minutes in the humidified CO<sub>2</sub> incubator

Collect cell supernatant (this can be stored at 4°C until ready for testing if necessary)

## **3. 17β-HSD3 DELFIA Assay Method**

Refer to DELFIA instruction manual

### **3.1 The protocol is summarised below.**

Pre-warm the required number of microtitration strips, DELFIA assay and wash buffer, Testosterone-Eu tracer, Testosterone antiserum, Testosterone standards and Enhancement solution.

Wash the microtitration strips with 1x 300 µl DELFIA wash buffer,

Add 25 µl of testosterone standards or 25 µl of cell supernatant,

Add 100 µl of 1 in 10 dilution of Testosterone-Eu tracer in DELFIA assay buffer,

Add 100 µl of 1 in 10 dilution of Testosterone antiserum in DELFIA assay buffer,

Seal plate and incubate at room temperature for 2 hours, shaking slowly,

Remove well contents and wash with 4x 300 µl DELFIA wash buffer,

Add 200 µl of Enhancement solution,

Seal plate and incubate at room temperature for 5minutes, shaking slowly,

Read on Victor V using Europium protocol,

Note:-It may be necessary to dilute the cell supernatant with media buffer prior to testing, to ensure that the levels of testosterone falls within the linear region of the Testosterone standard curve.

Testosterone standards are reconstituted in 1 mL media buffer rather than 1 mL distilled water.

### **3.2 Cell number estimation**

Following the removal of cell supernatant, after compound / substrate addition, replace with 100 µl of assay buffer



Add 20 µl of 1 in 20 dilution of PMS (phenazine methosulphate) solution, incubate for 60 minutes in the humidified CO<sub>2</sub> incubator. Read absorbance at 492 nm on a spectrophotometer (MultiSkan MCC/340)

### 3.3 Calculation of results

All measurements were performed in duplicate, testosterone concentrations produced by the cells are determined from testosterone standard curves which are plotted using a Spline/Lowess equation in Graphpad PRISM®.

The level of testosterone is corrected for cell number, using the absorbance values. Levels of testosterone in the presence of antagonist were expressed as percent inhibition of the total testosterone produced.

To determine enzyme activity and percentage inhibition:

First, put the raw data into a spreadsheet program (MS Excel),

Calculate the B/Bmax for each testosterone concentration for the standard curve,

(B/Bmax = RFU value of sample/RFU value of standard A (0 nM testosterone),

Calculate the B/Bmax for all the unknown samples,

In Graphpad Prism:

Plot the B/Bmax for the standards on the y-axis against the concentration of the standards against the x-axis,

Type in the B/Bmax of the unknown samples in the y-axis column, the data was analysed with a cubic spline curve fit and the x-value (testosterone level) of the unknown samples was calculated from the B/Bmax.

Copy the testosterone levels back into the spreadsheet program and, if samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor. Divide the corrected testosterone levels by the corresponding absorbance values at 492nm for the MTS assay to correct for cell number, calculate the percentage inhibition according to the formula:

$$100 - ((\text{Testosterone}_{\text{Sample}} - \text{Testosterone}_{\text{Blank}}) / (\text{Testosterone}_{\text{Control}} - \text{Testosterone}_{\text{Blank}})) \times 100$$

## Chapter 15

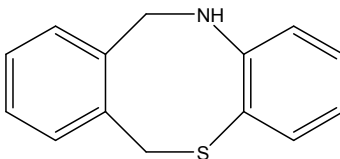
### Experimental Details: Chemistry

All chemicals were purchased from Aldrich Chemical Co. (Gillingham, UK) or Lancaster Synthesis (Morecambe, U.K.). All organic solvents were supplied by Fisher Scientific (Loughborough, U.K.). Reactions using anhydrous solvents were carried out under nitrogen.

Thin layer chromatography (TLC) was performed on precoated plates (Merck TLC aluminium sheets silica gel 60 F<sub>254</sub>). Product(s) and starting material(s) were detected by either viewing under UV light and/or treating with a suitable staining system, for example vanillin, followed by heating. Flash column chromatography was performed on silica gel (Sorbisil/Matrex C60) or using Argonaut prepacked columns with a Flashmaster<sup>TM</sup>. IR spectra were recorded on a Perkin-Elmer Spectrum RXI FT-IR in DCM solution cells and peak positions are expressed in cm<sup>-1</sup>. <sup>1</sup>H NMR (270 MHz or 400 MHz) and *DEPT*-edited <sup>13</sup>C NMR (68 MHz or 101 MHz) spectra were recorded with a Jeol Delta 270 or a Varian Mercury VX 400 NMR spectrometer and chemical shifts are reported in parts per million (ppm). HPLC analyses were performed on a Waters Millenium 32 instrument equipped with a Waters 996 PDA detector. A Waters Radialpack C18 reversed phase column (8×100 mm) was eluted with the solvent system specified at 1 mL/min. FAB low and high resolution mass spectra were recorded at the Mass Spectrometry Service Centre, University of Bath, using *m*-nitrobenzyl alcohol (NBA) as the matrix. ES and APCI low resolution mass spectra were obtained on a Waters Micromass ZQ. Elemental analyses were performed by the Microanalysis Service, University of Bath. Melting points were determined using a Reichert-Jung Thermo Galen Kofler block and are uncorrected.

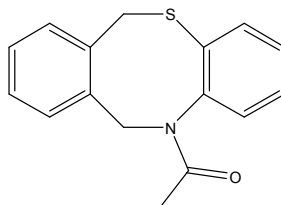
## Experimental Details: Chapter 2: Synthesis of Compound 26 as Positive Control

**6, 11-Dihydro-12H-dibenzo[*b,f*]1,4]thiazocine, 38**, C<sub>14</sub>H<sub>13</sub>NS, MW 227.32,



The above compound was synthesised by a modification of the method described by Yale *et al.*<sup>54</sup> To a solution of  $\alpha,\alpha'$ -dibromo-o-xylene (250 mg, 0.95 mmol) in DMF (5 mL) at 100 °C was added 2-aminothiophenol (0.1 mL, 0.95 mmol) in DMF (5 mL) and the resulting solution stirred at 100 °C for 10 min. The DMF was removed in-vacuo, then NaHCO<sub>3</sub> (20 mL) and DCM (2 x 20 mL) were added and the product extracted, evaporated to dryness and isolated as a yellow oil, 435 mg (>>100 %). This was used crude in following steps. R.f. 0.45 (Hexane: DCM, 1:1), LCMS:  $t_r$  = 2.46 min (80 % MeOH in water),  $m/z$  M+H 227.9, HPLC:  $t_r$  = 2.3 min (90 % acetonitrile in water), ~60 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  3.92 (1H, br.s, NH), 4.40 (2H, s, CH<sub>2</sub>), 4.85 (2H, s, CH<sub>2</sub>), 6.75-6.88 (2H, m, ArH), 7.10-7.45 (6H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): 36.6, 49.5 (CH<sub>2</sub>), 120.3, 120.5 (ArCH), 124.3 (ArC), 127.5, 127.6, 128.1, 129.0, 129.7, 133.4 (ArCH), 137.1, 139.6, 148.0 (ArC).

**12-Acetyl-6,11-dihydro-12H-dibenzo[*b,f*][1,4]thiazocine, 26**, C<sub>16</sub>H<sub>15</sub>NOS, MW 269.36,

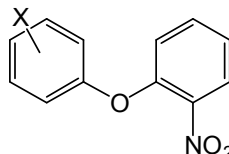


The above compound was synthesised by considerable modification of the procedure described by Yale *et al.*<sup>54</sup> 6,11-Dihydro-12H-dibenzo[*b,f*][1,4]thiazocine (215 mg, 0.95 mmol) in DCM (5 mL) was cooled to 0 °C and to this was added acetyl chloride (0.3 mL, 2 mmol) and TEA (0.15 mL, 2 mmol). The resulting solution was stirred at r.t. for 1 h. Saturated NaHCO<sub>3</sub> solution (20 mL) was added and this was extracted with DCM (2 x 20mL) and dried (MgSO<sub>4</sub>). The crude product was purified by flash chromatography (0-100 % DCM in hexane), 185 mg, 73% yield (over 2 steps). R.f. 0.35 (DCM:Hexane), m.p. 123-125 °C, (lit. 124-126 °C),<sup>54</sup> LCMS:  $t_r$  = 1.01min (95 % MeOH in water),  $m/z$  M+H 270.02, HPLC:  $t_r$  = 1.83 min (90 % acetonitrile in water), 96 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  1.87 (3H, s, CH<sub>3</sub>), 3.67 (1H, d,  $J$ =12.4,  $\frac{1}{2}$ CH<sub>2</sub>), 4.02 (1H, d,  $J$ = 15.1 Hz,  $\frac{1}{2}$ CH<sub>2</sub>), 4.45 (1H, d,  $J$ = 12.4 Hz,  $\frac{1}{2}$ CH<sub>2</sub>), 6.66 (1H, d,  $J$ = 15.1 Hz,  $\frac{1}{2}$ CH<sub>2</sub>), 6.90-7.25 (8H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): 22.9 (CH<sub>3</sub>), 34.7, 51.1 (CH<sub>2</sub>), 127.6, 128.2, 128.7, 128.8, 129.4, 130.2, 132.4 (ArCH), 134.7, 135.1, 135.7,

140.9 (ArC), 170.6 (CO). HRMS: Calcd for C<sub>16</sub>H<sub>15</sub>NOS (M+H)<sup>+</sup> 292.075 (+Na), found (M+H)<sup>+</sup> 292.0767 (+Na).

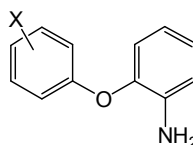
## Experimental Details: Chapter 6: Synthesis and Biological Evaluation of the Amide Linked Series of Inhibitors

### General Procedure for the 2-Nitro-diphenylether Formation



This procedure is modified from that described by Matsuo *et al.*<sup>65</sup> A mixture of 2-fluoro-1-nitrobenzene (1 eq.), substituted phenol (1.5 eq.) and potassium carbonate (1.5 eq.) in DMF (5 mmol/mL) was stirred at reflux for 3 to 19 h. The solvent was removed *in vacuo* and the residue was dissolved in diethylether and washed with sodium hydroxide (5 %). The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo* to afford the desired 2-nitro-diphenylether. Flash column chromatography was carried out if required.

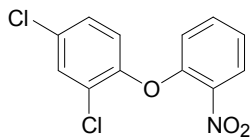
### General Procedure for the Reduction of the Nitro Group



This procedure is modified from that described by Matsuo *et al.*<sup>65</sup> To a mixture of iron (5.5 eq.) and ammonium chloride (0.7 eq.) in a 10:1 mixture of EtOH: H<sub>2</sub>O, the 2-nitro-diphenylether (1 eq.) was added. This reaction mixture was stirred at reflux for 30 min-4 h. The reaction was monitored by TLC, until complete consumption of the starting material was observed. It was allowed to cool and the solvent removed *in vacuo*. The residue was re-dissolved in DCM and washed with sat. NaHCO<sub>3</sub>. The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo* to afford the desired diphenylether aniline. Flash column chromatography was carried out if required.

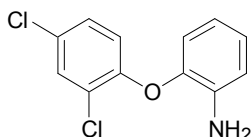
## Synthesis of Diphenylether Anilines

**1-(2, 4-Dichlorophenoxy)-2-nitrobenzene, 40a**, C<sub>12</sub>H<sub>7</sub>Cl<sub>2</sub>NO<sub>3</sub>, MW 284.09,



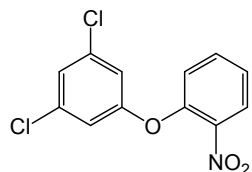
Using the general procedure for the 2-nitro-diphenylether formation the desired compound was obtained as a light yellow solid, 3.4 g, 97 % yield. R.f: 0.73 (DCM), m.p. 54-55 °C. HPLC  $t_r$  = 2.39 min (90% acetonitrile in H<sub>2</sub>O) 99 %, LCMS  $m/z$  M-H 283.06, 285.07, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  6.85 (1H, dd,  $J$  = 8.4, 1.2 Hz, ArH), 6.97 (1H, d,  $J$  = 8.9 Hz, ArH), 7.23 (2H, m, ArH), 7.48 (1H, d,  $J$  = 2.5 Hz, ArH), 7.51 (1H, m, ArH) and 7.98 (1H, dd,  $J$  = 8.2, 1.7 Hz, ArH).

**2-(2, 4-Dichlorophenoxy)phenylamine, 41a**, C<sub>12</sub>H<sub>9</sub>Cl<sub>2</sub>NO, MW 254.11,



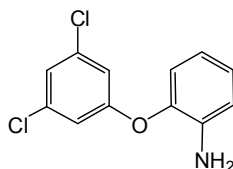
Using the general procedure for the reduction of the nitro group the desired product was obtained as an oil, 2.01 g, 90 % yield. R.f: 0.56 (DCM), HPLC  $t_r$  = 2.51 min (90% acetonitrile in H<sub>2</sub>O) 93 %, LCMS  $m/z$  M+H 254.27, 256.29, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  3.85 (2H, br s, NH<sub>2</sub>), 6.75 (1H, m, ArH), 6.84 (1H, d,  $J$  = 8.8 Hz, ArH), 6.84 (1H, dd, ArH), 6.87 (1H, dd,  $J$  = 7.6, 1.6 Hz, ArH), 7.04 (1H, m, ArH), 7.17 (1H, dd,  $J$  = 8.8, 2.4 Hz, ArH) and 7.49 (1H, d,  $J$  = 2.4 Hz, ArH).

**1,3-Dichloro-5-(2-nitro-phenoxy)benzene, 40b**, C<sub>12</sub>H<sub>7</sub>Cl<sub>2</sub>NO<sub>3</sub>, MW 284.10,



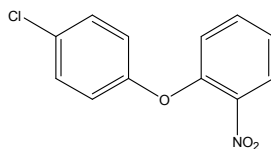
Using the general procedure for the 2-nitro-diphenylether formation the desired product was obtained as a light yellow solid, 0.57 g, 33 % yield. m.p. 78-80 °C, (lit. 77-79 °C)<sup>65</sup> <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  6.88 (2H, d,  $J$  = 1.7 Hz, ArH, ArH), 7.12 (1H, t,  $J$  = 1.7 Hz, ArH), 7.12 (1H, dd,  $J$  = 1.2, 8.2 Hz, ArH), 7.32 (1H, td,  $J$  = 7.4, 1.2 Hz, ArH), 7.6 (1H, td,  $J$  = 7.4, 1.7 Hz, ArH), 8.00 (1H, dd,  $J$  = 8.2, 1.7 Hz, ArH).

**2-(3,5-Dichloro-phenoxy)phenylamine, 41b**, C<sub>12</sub>H<sub>9</sub>Cl<sub>2</sub>NO, MW 254.11,



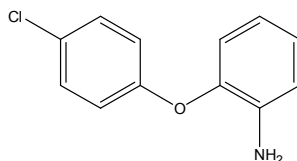
Using the general procedure for the reduction of the nitro group the desired compound was obtained as a brown oil, 1.45 g, 89 % yield. R.f 0.6 (DCM); LCMS:  $t_r$  = 3.01 min (90 % MeOH in water),  $m/z$  M+H 254.1, 256.2, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  3.67 (2H, s, NH<sub>2</sub>), 6.68 (1H, td,  $J$  = 7.4, 1.5 Hz, ArH), 6.77 (1H, dd,  $J$  = 7.9, 1.5 Hz, ArH), 6.77 (2H, d,  $J$  = 2.0 Hz, ArH, ArH), 6.84 (1H, dd,  $J$  = 7.9, 1.5 Hz, ArH), 6.97 (1H, t,  $J$  = 2.0 Hz, ArH), 6.98 (1H, td,  $J$  = 7.4, 1.5 Hz, ArH). HRMS: Calcd for C<sub>12</sub>H<sub>9</sub>Cl<sub>2</sub>NO (M+H)<sup>+</sup> 254.0134, found (M+H)<sup>+</sup> 254.0124. Compound has been previously synthesised using the same method by Matsuo *et al.*<sup>65</sup>

**2-(4-Chloro-phenoxy)nitrobenzene, 40c**, C<sub>12</sub>H<sub>8</sub>ClNO<sub>3</sub>, MW 249.65.<sup>135</sup> (Alternative MW Method),



4-Chlorophenol (2.9 g, 22.5 mmol) and 2-fluoronitrobenzene (1.6 mL, 15 mmol) was dissolved in toluene (30 mL) and then placed into 10 separate MW tubes (3 mL solution/tube). To each tube was then added K<sub>2</sub>CO<sub>3</sub> (373 mg, 2.7 mmol). Each tube was then subjected to MW heating at 180 °C for 30 min. All tubes were combined, the solvent was removed *in vacuo* and the residue was dissolved in diethylether (100 mL) and washed with sodium hydroxide (5 %, 50 mL). The organic layers were combined, dried (MgSO<sub>4</sub>), filtered, and evaporated *in vacuo* to afford the desired product as a wax. Purification was not carried out so the product was used crude in the next step. A small sample was purified to allow for characterisation. HPLC  $t_r$  = 5.4 min (90 % acetonitrile in H<sub>2</sub>O) 95 %, LCMS  $m/z$  M-H 248.11, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  6.92-6.97 (2H, m, ArH), 7.00 (1H, dd,  $J$  = 1.2, 8.4 Hz, ArH), 7.19-7.25 (1H, m, ArH), 7.28-7.33 (2H, m, ArH), 7.48-7.55 (1H, m, ArH), 7.93 (1H, dd,  $J$  = 1.5, 8.2 Hz, ArH).

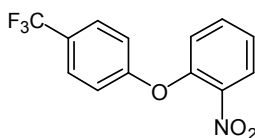
**2-(4-Chloro-phenoxy)phenylamine, 41c**, C<sub>12</sub>H<sub>10</sub>ClNO, MW 219.67,



Using the general procedure for the reduction of the nitro group and subsequent purification (0-50 % DCM in hexane) the desired product was obtained as a light yellow oil, 2.7 g (82 % yield over 2 steps). R.f. 0.55 (EtOAc), LCMS:  $t_r$  = 4.87 min (90 %

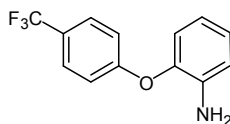
MeOH in water),  $m/z$  M+H 219.9, HPLC:  $t_r$  = 2.12 min (90 % acetonitrile in water), 94 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  3.78 (2H, br.s, NH), 6.67-6.76 (1H, m, ArH), 6.81-6.92 (4H, m, ArH), 6.97-7.03 (1H, m, ArH), 7.22-7.27 (2H, m, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz):  $\delta$  116.8, 118.4, 119.1, 120.4, 125.4 (ArCH), 127.7 (ArC), 129.7 (ArCH), 138.71, 142.8, 156.2 (ArC). HRMS: Calcd for  $\text{C}_{12}\text{H}_{10}\text{ClNO}$  (M+H) $^+$  220.0524, found (M+H) $^+$  220.0531, 222.0487.

**4-Trifluoromethyl-5-(2-nitro-phenoxy)benzene, 40d**,  $\text{C}_{13}\text{H}_8\text{F}_3\text{NO}_3$ , MW 283.21,



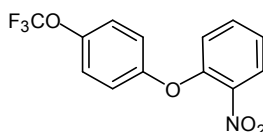
Using the general procedure for the 2-nitro-diphenylether formation the desired compound was obtained as a brown oil, 1.24 g, 68 % yield. R.f. 0.6 (1:1, DCM: Petrol),  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.11 (2H, d,  $J$  = 8.4 Hz, ArH), 7.17 (1H, dd,  $J$  = 8.4, 0.8 Hz, ArH), 7.36 (1H, td,  $J$  = 7.2, 1.2 Hz, ArH), 7.62-7.67 (1H, m, ArH), 7.65 (2H, d,  $J$  = 9.6 Hz, ArH), 8.04 (1H, dd,  $J$  = 1.6, 8.0 Hz, ArH). HRMS: Calcd for  $\text{C}_{13}\text{H}_8\text{F}_3\text{NO}_3$  (M+H) $^+$  284.0529, found (M+H) $^+$  284.0524.

**2-(4-Trifluoromethyl-phenoxy)phenylamine, 41d**,  $\text{C}_{13}\text{H}_{10}\text{F}_3\text{NO}$ , MW 253.23,



Using the general procedure for the reduction of the nitro group the desired compound was obtained as a light yellow oil, 0.97 g, 82 % yield. R.f. 0.45 (DCM: hexane, 1:1); LCMS:  $t_r$  = 2.11 min (90 % MeOH in water),  $m/z$  M+H 254.2,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  3.84 (2H, s,  $\text{NH}_2$ ), 6.84 (1H, td,  $J$  = 7.2, 1.2 Hz, ArH), 6.91 (1H, dd,  $J$  = 1.6, 8.0 Hz, ArH), 7.01 (1H, dd,  $J$  = 0.8, 7.6 Hz, ArH), 7.10 (2H, d,  $J$  = 9.2 Hz, ArH), 7.13 (1H, td,  $J$  = 4.0, 7.2 Hz, ArH), 7.63 (2H, d,  $J$  = 8.4 Hz, ArH). HRMS: Calcd for  $\text{C}_{13}\text{H}_{10}\text{F}_3\text{NO}$  (M+H) $^+$  254.0787, found (M+H) $^+$  254.0776. This compound has been previously synthesised using a different methods by Golinske *et al.*<sup>136</sup>

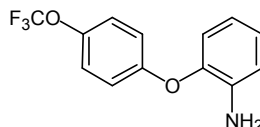
**1-Trifluoromethoxy-4-(2-nitro-phenoxy)benzene, 40e**,  $\text{C}_{13}\text{H}_8\text{F}_3\text{NO}_4$ , MW 299.21,



Using the general procedure for the 2-nitro-diphenylether formation the desired compound was obtained as a yellow oil, 2.34 g, >100 % yield. R.f. 0.72 (DCM),  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  7.01-7.06 (3H, m, ArH), 7.20 (2H, dd,  $J$  = 0.9, 10.1 Hz,

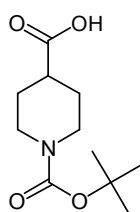
ArH), 7.21-7.27 (1H, m, ArH), 7.51-7.54 (1H, m, ArH), 7.97 (1H, dd,  $J = 1.5, 8.2$  Hz, ArH). This compound was previously synthesised using a different method, by McEvoy *et al.*<sup>137</sup>

**2-(4-Trifluoromethoxy-phenoxy)phenylamine, 41e**, C<sub>13</sub>H<sub>10</sub>F<sub>3</sub>NO<sub>2</sub>, MW 269.22,<sup>137</sup>



Using the general procedure for the reduction of the nitro group the desired compound was obtained as a brown oil, 1.57 g, 95 % yield. R.f: 0.6 (DCM), <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  3.81 (2H, s, NH<sub>2</sub>), 6.76 (1H, td,  $J = 1.5, 8.2$  Hz, ArH), 6.84 (1H, dd,  $J = 1.2, 7.9$  Hz, ArH), 6.91 (1H, dd,  $J = 1.0, 7.9$  Hz, ArH), 6.98 (2H, d,  $J = 9.1$  Hz, ArH), 7.01-7.07 (1H, m, ArH), 7.18 (2H, d,  $J = 8.7$  Hz, ArH). This compound was previously synthesised using a different method, by McEvoy *et al.*<sup>137</sup>

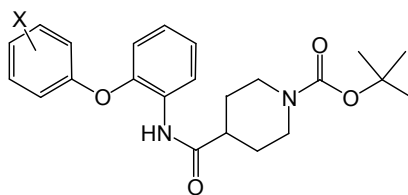
**N-Boc-piperidine-4-carboxylic acid, 42**, C<sub>11</sub>H<sub>19</sub>NO<sub>4</sub>, MW 229.28,



To a solution of isonipecotic acid (2.0 g, 16 mmol) in 1,4-dioxane (50 mL) and water (50 mL) was added di-*t*-butyl dicarbonate (3.4 g, 16 mmol) and sodium hydroxide (6.2 g, 155 mmol). The reaction mixture was stirred at room temperature for 21 h. The solvent was removed *in vacuo* and the residue was re-dissolved in EtOAc and acidified to pH 3-4 using hydrochloric acid (1M). The mixture was then extracted with further EtOAc (100 mL) and washed with water (50 mL). The organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo* to afford the title compound as a white solid, 2.2 g, 61 % yield. m.p. 151-153 °C, (Lit. 148-153 °C),<sup>138</sup> R.f: 0.72 (10 % MeOH in DCM), LCMS:  $t_r = 2.13$  min (90 % MeOH in water),  $m/z$  M+Na 252.2, <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>):  $\delta$  1.4 (9H, s, CH<sub>3</sub>), 1.55-1.69 (2H, m, CH<sub>2</sub>), 1.89 (2H, dd,  $J = 3.0, 13.4$  Hz, CH<sub>2</sub>), 2.42-2.53 (1H, m, CH), 2.83 (2H, t,  $J = 11.1$  Hz, N-CH<sub>2</sub>), 4.03 (2H, d,  $J = 12.0$  Hz, N-CH<sub>2</sub>). HRMS: Calcd for C<sub>11</sub>H<sub>19</sub>NO<sub>4</sub> (M+Na)<sup>+</sup> 252.1206, found (M+Na)<sup>+</sup> 252.1199.

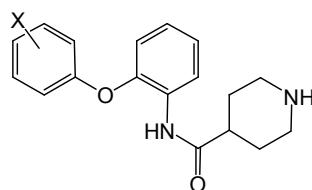


### General Procedure for the Formation of the Amide Linker between the Diphenylether Aniline and Piperidine Units



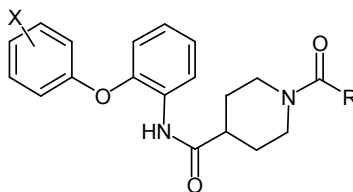
This method is adapted from a method by Vicker *et al.*<sup>139</sup> To a solution of *N*-Boc-piperidine-4-carboxylic acid (1 eq.) in anhydrous DCM (0.15 mmol/mL), was added DMAP (cat.), EDC (3.5 eq.) and TEA (0.15 mL/mmol). The reaction mixture was stirred under nitrogen at room temperature for 30 min. The diphenylether aniline (1.2 eq.) was then added and the mixture was stirred under nitrogen for a further 18-96 h. It was then diluted with DCM and washed with hydrochloric acid (1M), NaHCO<sub>3</sub> and finally, brine. The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography to afford the desired product.

### General Procedure for the Removal of the *N*-Boc Protecting Group



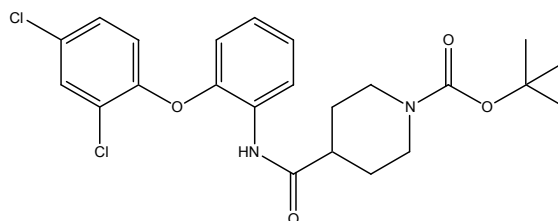
To a solution of the desired *t*-Boc-protected amine (1 eq.) in anhydrous DCM (10 mL/1 mmol) at 0 °C was added TFA (5 mL/1 mmol). The reaction mixture was stirred under nitrogen for 1-2 h, poured onto solid K<sub>2</sub>CO<sub>3</sub> (12 g/mmol) and water was added. The resulting solution was extracted with DCM and the organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo* to afford the desired amine.

### General Procedure for the Acylation of the Piperidine Nitrogen



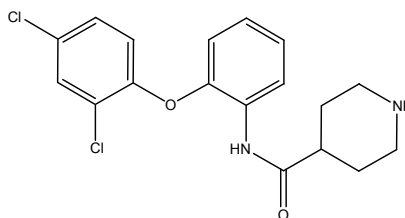
To a solution of the desired amine (1 eq.) in DCM (5 mL/2 mmol) at 0 °C, was added acid chloride (2 eq.) and TEA (5 eq.). The reaction mixture was allowed to warm to room temperature and stirred for 1 h. The reaction was then quenched with sodium bicarbonate and extracted with DCM. The organic portions were washed with 1M HCl and finally, brine. The organic layers were dried (MgSO<sub>4</sub>) and evaporated *in vacuo*. The crude mixture was purified using flash chromatography to afford the desired acetylated product.

**4-[2-(2,4-Dichlorophenoxy)phenylcarbamoyl]piperidine-1-carboxylic acid *tert*-butyl ester, 43**, C<sub>23</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>4</sub>, MW 465.37,



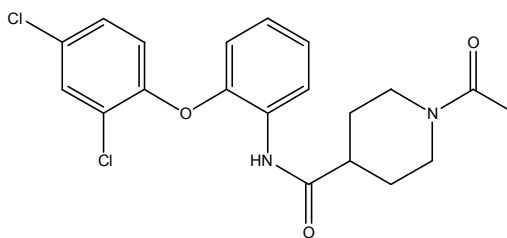
Using the general procedure for the formation of the amide linker, the desired compound was obtained as a white solid, which was used in the next step without further purification, 754 mg, 84 % yield. A small sample was purified for characterisation. m.p. 120-121 °C (from hexane), R.f: 0.31 (Hexane/EtOAc 7:3), LC/MS  $t_r$  = 1.39 min,  $m/z$  M-H 464.96, <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  1.44 (9H, s, 3CH<sub>3</sub>), 1.65-1.76 (2H, m, CH<sub>2</sub>), 1.84-1.86 (2H, br d, CH<sub>2</sub>), 2.40 (1H, tt, CH), 2.77 (2 H, br t, CH<sub>2</sub>), 4.14 (2H, br d, CH<sub>2</sub>), 6.71 (1H, dd,  $J$  = 8.2, 1.7 Hz, ArH), 6.94 (1H, d,  $J$  = 8.9 Hz, ArH), 7.01 (1H, td,  $J$  = 8.2, 1.5, ArH), 7.12 (1H, td,  $J$  = 8.2, 1.5 Hz, ArH), 7.21 (1H, dd,  $J$  = 8.9, 2.5 Hz, ArH), 7.48 (1H, d,  $J$  = 2.5 Hz, ArH), 7.76 (1H, br s, NH) and 8.40 (1H, dd,  $J$  = 7.9, 1.5 Hz, ArH). HRMS: Calcd for C<sub>23</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>4</sub> (M+H)<sup>+</sup> 465.1342, found (M+H)<sup>+</sup> 465.1333.

**Piperidine-4-carboxylic acid [2-(2,4-dichlorophenoxy)phenyl]amide, 40a**, C<sub>18</sub>H<sub>18</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>, MW 365.25,



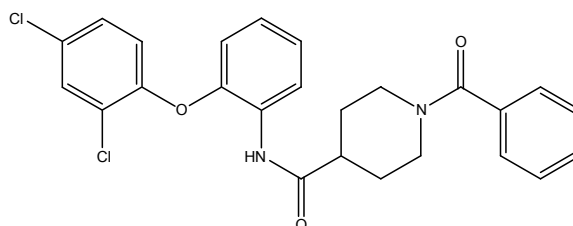
Using the general procedure for the removal of the *N*-Boc protecting group the desired compound was obtained as an oil which was used in the next step without further purification, 61 mg, 90 % yield. R.f: 0.175 (DCM/methanol 4:1 plus 3 drops of TEA), <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  1.65-1.80 (2H, m, CH<sub>2</sub>), 1.87-1.91 (2H, m, CH<sub>2</sub>), 2.24 (1H, br s, NH), 2.40 (1H, m, CH), 2.66 (2H, td, CH<sub>2</sub>), 3.15 (2H, br d,  $J$  = 12.6 Hz, CH<sub>2</sub>), 6.72 (1H, dd,  $J$  = 8.2, 1.5 Hz, ArH), 6.93 (1H, d,  $J$  = 8.6 Hz, ArH), 6.99 (1H, td,  $J$  = 7.9, 1.5 Hz, ArH), 7.12 (1H, td,  $J$  = 7.9, 1.2 Hz, ArH), 7.20 (1H, dd,  $J$  = 8.9, 2.5 Hz, ArH), 7.47 (1H, d,  $J$  = 2.5 Hz, ArH) and 8.41 (1H, dd,  $J$  = 7.9, 1.2 Hz, ArH).

**1-Acetylpiperidine-4-carboxylic acid [2-(2,4-dichlorophenoxy) phenyl]amide, 49,**  
 $C_{20}H_{20}Cl_2N_2O_3$ , MW 407.29,



To an ice cooled solution of piperidine-4-carboxylic acid [2-(2,4-dichlorophenoxy)phenyl]-amide (61 mg, 0.167 mmol) in dry DCM (6 mL) were added TEA (0.23 mL, 1.67 mmol) and acetyl chloride (0.024 mL, 0.334 mmol). The reaction mixture was stirred at room temperature until completion by TLC (30 min), and quenched with saturated  $NaHCO_3$  (20 mL). The resulting solution was extracted with DCM (3 x 20 mL) and the combined organic layers were washed with water, 1M HCl (3 x 20 mL) and brine, dried ( $MgSO_4$ ), filtered and evaporated to dryness. Flash chromatography of the crude product (hexane to EtOAc) gave the desired product as a white solid, 51 mg, 75 % yield. mp 194-196 °C, R.f: 0.15 (EtOAc), LC/MS (APCI) tr = 2.45 min, m/z M+H 409.31, HPLC tr = 2.594 min (99 %),  $^1H$  NMR (270 MHz,  $CDCl_3$ )  $\delta$  1.65-1.76 (2H, m,  $CH_2$ ), 1.89-1.92 (2H, m,  $CH_2$ ), 2.09 (3H, s,  $CH_3$ ), 2.51 (1H, m, CH), 2.67 (1H, m,  $\frac{1}{2}CH_2$ ), 3.11 (1H, m,  $\frac{1}{2}CH_2$ ), 3.87 (1H, m,  $\frac{1}{2}CH_2$ ), 4.59 (1H, m,  $\frac{1}{2}CH_2$ ), 6.72 (1H, dd,  $J$  = 8.0, 1.5 Hz, ArH), 6.94 (1H, d,  $J$  = 8.9 Hz, ArH), 7.00 (1H, td, ArH), 7.12 (1H, td, ArH), 7.22 (1H, dd,  $J$  = 8.7, 2.5 Hz, ArH), 7.48 (1H, d,  $J$  = 2.5 Hz, ArH), 7.79 (1H, br s, NH) and 8.38 (1H, br d,  $J$  = 8.2 Hz, ArH). HRMS: Calcd for  $C_{20}H_{20}Cl_2N_2O_3$  (M+H) $^+$  407.0924, found (M+H) $^+$  407.0924.

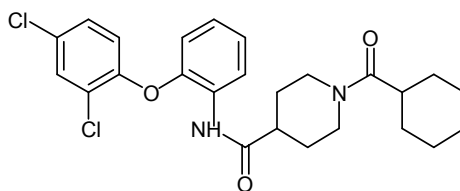
**1-Benzoylpiperidine-4-carboxylic acid [2-(2,4-dichloro-phenoxy)phenyl] amide, 50,**  
 $C_{25}H_{22}Cl_2N_2O_3$ , MW 469.36,



To an ice cooled solution of piperidine-4-carboxylic acid [2-(2,4-dichlorophenoxy)phenyl]-amide (100 mg, 0.274 mmol) in dry DCM (10 mL) were added pyridine (0.044 mL, 0.548 mmol) and benzoyl chloride (0.047 mL, 0.411 mmol). The reaction mixture was stirred at room temperature until TLC showed consumption of starting material (1 h), then quenched with saturated  $NaHCO_3$  (15 mL). The resulting solution was extracted with DCM (3 x 20 mL) and the combined organic layers were washed with water, 1M HCl (3 x 20 mL) and brine (20 mL), dried ( $MgSO_4$ ), filtered and evaporated. Flash chromatography of the crude product (hexane to EtOAc) gave

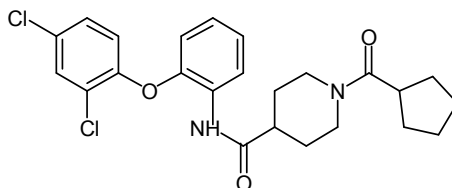
the desired product as a white solid, 98 mg, 76 % yield. mp 176-177 °C, R.f: 0.17 (hexane/EtOAc 1:1), LC/MS (APCI)  $t_r$  = 1.10 min,  $m/z$  M+H 471.26, HPLC  $t_r$  = 2.88 min (99 %),  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ )  $\delta$  1.85 (4H, m, 2CH<sub>2</sub>), 2.55 (1H, tt, CH), 3.01 (2H, m, CH<sub>2</sub>), 3.89 (1H, m,  $\frac{1}{2}\text{CH}_2$ ), 4.74 (1H, m,  $\frac{1}{2}\text{CH}_2$ ), 6.72 (1H, dd,  $J$  = 8.2, 1.5 Hz, ArH), 6.95 (1H, d,  $J$  = 8.6 Hz, ArH), 7.01 (1H, td, ArH), 7.14 (1H, td, ArH), 7.22 (1H, dd,  $J$  = 8.6, 2.5 Hz, ArH), 7.39 (5H, s, ArH), 7.49 (1H, d,  $J$  = 2.5 Hz, ArH), 7.78 (1H, br s, NH) and 8.38 (1H, br d,  $J$  = 8.2, ArH). Anal. Calcd. for  $\text{C}_{25}\text{H}_{22}\text{Cl}_2\text{N}_2\text{O}_3$ : C 63.97, H 4.72, N 5.97. Found: C 64.10, H 4.83, N 5.88 %.

**1-Cyclohexanecarbonyl-piperidine-4-carboxylic acid [2-(2,4-dichloro-phenoxy)-phenyl]-amide, 51**,  $\text{C}_{25}\text{H}_{25}\text{Cl}_2\text{N}_2\text{O}_3$ , MW 475.41



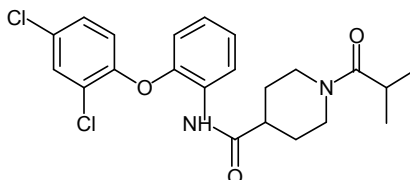
To a solution of piperidine-4-carboxylic acid [2-(2,4-dichloro-phenoxy)-phenyl]-amide (0.1 g, 0.3 mmol) in DCM (5 mL) at 0 °C was added TEA (0.2 mL) and cyclohexanecarbonyl chloride (0.072 mL, 0.54 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction was quenched with sat.  $\text{NaHCO}_3$  (15 mL), extracted with DCM (2 x 20 mL), washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried ( $\text{MgSO}_4$ ), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-5 % methanol in DCM), to afford a white solid, 120 mg, 95 % yield. m.p. 46-48 °C, R.f. 0.65 (EtOAc), LCMS:  $t_r$  = 5.42 min (50 % MeOH in water at 0.5 mL/min),  $m/z$  M+H 475.16, HPLC:  $t_r$  = 4.24 min (80 % acetonitrile in water at 1.0 mL/min), 97 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.14-1.89 (14H, m, 7CH<sub>2</sub>), 2.23-2.32 (1H, m, CH), 2.33-2.49 (1H, m, CH), 2.61 (1H, t,  $J$  = 10.0 Hz, CH<sub>2</sub>), 3.02 (1H, t,  $J$  = 9.5 Hz, CH<sub>2</sub>), 3.92 (1H, d,  $J$  = 11.3 Hz, CH<sub>2</sub>), 4.57 (1H, d,  $J$  = 11.9 Hz, CH<sub>2</sub>), 6.67 (1H, dd,  $J$  = 7.3, 1.4 Hz, ArH), 6.89 (1H, d,  $J$  = 7.8 Hz, ArH), 6.95 (1H, td,  $J$  = 7.0, 1.4 Hz, ArH), 7.07 (1H, td,  $J$  = 1.4, 7.6 Hz, ArH), 7.16 (1H, dd,  $J$  = 2.4, 8.1 Hz, ArH), 7.43 (1H, d,  $J$  = 2.2 Hz, ArH), 7.69 (1H, s, NH), 8.34 (1H, d,  $J$  = 7.02 Hz, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta$  25.8, 25.9, 29.3, 29.5 (CH<sub>2</sub>), 40.5 (CH), 41.0 (CH<sub>2</sub>), 44.4 (CH), 44.7 (CH<sub>2</sub>), 116.5, 121.4, 121.5, 124.2, 124.6 (ArCH), 126.4 (ArC), 128.4 (ArCH), 128.9, 130.1 (ArC), 126.4 (ArC), 128.4 (ArCH), 128.9, 130.1 (ArC), 130.7 (ArCH), 145.2, 150.3 (ArC), 172.2, 174.6 (CO). HRMS: Calcd for  $\text{C}_{25}\text{H}_{25}\text{Cl}_2\text{N}_2\text{O}_3$  (M+H)<sup>+</sup> 475.1550, found (M+H)<sup>+</sup> 475.1552,

**1-Cyclopentanecarbonyl-piperidine-4-carboxylic acid [2-(2,4-dichloro-phenoxy)-phenyl]-amide, 52**, C<sub>24</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>, MW 461.38



To a solution of piperidine-4-carboxylic acid [2-(2,4-dichloro-phenoxy)-phenyl]-amide (0.1 g, 0.3 mmol) in DCM (5 mL) at 0 °C was added TEA (0.2 mL) and cyclopentane carbonyl chloride (0.066 mL, 0.54 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction was quenched with sat. NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL), washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % DCM in hexane). The desired product was recrystallised from diethyl ether/hexane as a white solid, 46 mg, 37 % yield. m.p. 135-137 °C, R.f. 0.67 (EtOAc), LCMS: *t*<sub>r</sub> = 5.29 min (50 % MeOH in water at 0.5 mL/min), *m/z* M+H 461.32, HPLC: *t*<sub>r</sub> = 3.96 min (80 % acetonitrile in water at 1.0 mL/min), 98 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.54-1.97 (12H, m, 6CH<sub>2</sub>) 2.46-2.57 (1H, m, CH), 2.63-2.73 (1H, m, CH<sub>2</sub>), 2.82-2.93 (1H, m, CH), 3.03-3.08 (1H, m, CH<sub>2</sub>), 4.03 (1H, d, *J* = 13.8 Hz, CH<sub>2</sub>), 4.63 (1H, d, *J* = 13.1 Hz, CH<sub>2</sub>), 6.72 (1H, dd, *J* = 8.15, 1.46 Hz, ArH), 6.95 (1H, d, *J* = 8.7 Hz, ArH), 7.00 (1H, td, *J* = 7.6, 1.73 Hz, ArH), 7.13 (1H, td, *J* = 7.91, 1.2 Hz, ArH), 7.22 (1H, dd, *J* = 8.64, 2.48 Hz, ArH), 7.49 (1H, d, *J* = 2.5 Hz, ArH), 7.76 (1H, s, NH), 8.4 (1H, dd, *J* = 8.15 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 26.0, 28.6, 29.1, 29.9, 30.2 (CH<sub>2</sub>), 41.1 (CH), 41.2 (CH<sub>2</sub>), 44.4 (CH), 44.8 (CH<sub>2</sub>), 116.5, 121.4, 121.4, 124.2, 124.6 (ArCH), 126.31 (ArC), 128.3 (ArCH), 128.8, 130.1 (ArC), 130.6 (ArCH), 145.1, 150.3 (ArC), 172.3, 174.4 (CO). HRMS: Calcd for C<sub>24</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 461.1393, found (M+H)<sup>+</sup> 461.1385. Anal. Calcd for C<sub>24</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>: C 62.48, H 5.68, N 6.07 %. Found C 62.5, H 5.63, N 5.96 %.

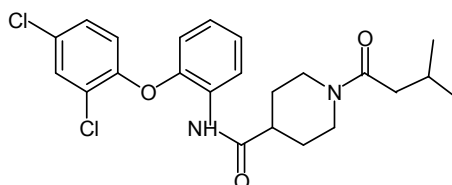
**1-Isobutyryl-piperidine-4-carboxylic acid [2-(2,4-dichloro-phenoxy)-phenyl]-amide, 53**, C<sub>22</sub>H<sub>24</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>, MW 435.34,



To a solution of piperidine-4-carboxylic acid [2-(2,4-dichloro-phenoxy)-phenyl]-amide (0.1 g, 0.3 mmol) in DCM (5 mL) at 0 °C was added TEA (0.2 mL) and isobutyryl chloride (0.057 mL, 0.54 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction was quenched with sat. NaHCO<sub>3</sub> (15

mL), extracted with DCM (2 x 20 mL), washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-10 % methanol in DCM). The title compound was recrystallised from diethylether/hexane as a white solid, 89 mg, 75 % yield. m.p. 50-52 °C, R.f. 0.65 (EtOAc), LCMS:  $t_r$  = 5.0 min (50 % MeOH in water at 1.0 mL/min),  $m/z$  M+H 435.31, HPLC:  $t_r$  = 2.58 min (90 % acetonitrile in water at 1.0 mL/min), 97 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.05-1.06 (6H, m, CH<sub>3</sub>), 1.61-1.70 (2H, m, CH<sub>2</sub>), 1.88 (2H, s, CH<sub>2</sub>), 2.41-2.56 (1H, m, CH), 2.59-2.69 (1H, m, CH<sub>2</sub>), 2.71-2.78 (1H, m, CH), 3.04 (1H, t,  $J$  = 11.1 Hz, CH<sub>2</sub>), 3.94 (1H, d,  $J$  = 12.2 Hz, CH<sub>2</sub>), 4.58 (1H, d,  $J$  = 11.6 Hz, CH<sub>2</sub>), 6.67 (1H, td,  $J$  = 6.2, 1.4 Hz, ArH), 6.89 (1H, d,  $J$  = 8.1 Hz, ArH), 6.95 (1H, td,  $J$  = 6.8 Hz, ArH), 7.07 (1H, td,  $J$  = 7.29, 1.1 Hz, ArH), 7.16 (1H, dd,  $J$  = 7.83, 2.16 Hz, ArH), 7.43 (1H, d,  $J$  = 2.2 Hz, ArH), 7.71 (1H, s, NH), 8.30 (1H, dd,  $J$  = 7.29, 1.1 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 19.3, 19.6 (CH<sub>3</sub>), 28.6, 29.1 (CH<sub>2</sub>), 30.10 (CH), 41.1 (CH<sub>2</sub>), 44.30 (CH), 44.7 (CH<sub>2</sub>), 116.4, 121.3, 121.42, 124.2, 124.6, 126.34, 128.40, 128.81, 130.20, 130.70, 145.11, 150.30 (ArC), 172.2, 175.30 (C=O). HRMS: Calcd for C<sub>22</sub>H<sub>24</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 435.1237, found (M+H)<sup>+</sup> 435.1233. Anal. Calcd for C<sub>22</sub>H<sub>24</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>: C 60.70, H 5.56, N 6.43 %. Found C 61.0, H 5.71, N 6.38 %.

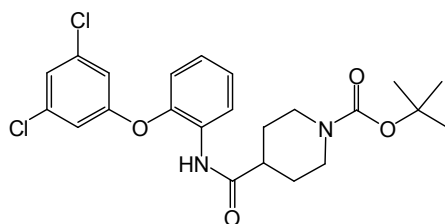
**1-(3-Methyl-butyl)-piperidine-4-carboxylic acid [2-(2,4-dichloro-phenoxy)-phenyl]-amide, 54**, C<sub>23</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>, MW 449.37



To a solution of piperidine-4-carboxylic acid [2-(2,4-dichloro-phenoxy)-phenyl]-amide (0.1 g, 0.3 mmol) in DCM (5 mL) at 0 °C was added TEA (0.2 mL) and isovaleryl chloride (0.057 mL, 0.54 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction was quenched with sat. NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL), washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-10 % methanol in DCM) to afford the title compound as a white waxy solid, 80 mg, 65 % yield. m.p. 60-62 °C, R.f. 0.65 (EtOAc), LCMS:  $t_r$  = 5.08 min (50 % MeOH in water at 0.5 mL/min),  $m/z$  M+H 449.35, HPLC:  $t_r$  = 2.69 min (90 % acetonitrile in water at 1.0 mL/min), 95 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 0.73 (6H, d,  $J$  = 5.9 Hz, 2CH<sub>3</sub>), 1.40-1.57 (2H, m, CH<sub>2</sub>), 1.68-1.72 (2H, m, CH<sub>2</sub>), 1.80-1.93 (1H, m, CH), 1.96-2.07 (3H, m, CH<sub>2</sub>, CH), 2.22-2.32 (1H, m, CH), 2.45 (1H, td,  $J$  = 12.4, 2.4 Hz, CH<sub>2</sub>), 2.85 (1H, td,  $J$  = 12.4, 2.16 Hz, CH<sub>2</sub>), 3.70 (1H, dd,  $J$  = 12.2, CH<sub>2</sub>), 4.40 (1H, d,  $J$  = 11.9 Hz, CH<sub>2</sub>),

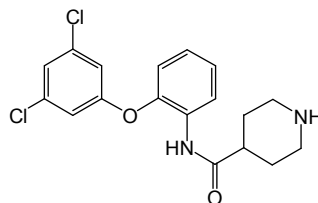
6.49 (1H, dd,  $J = 7.3, 1.1$  Hz, ArH), 6.72 (1H, d,  $J = 7.8$  Hz, ArH), 6.77 (1H, td,  $J = 6.8, 1.4$  ArH), 6.90 (1H, td,  $J = 7.3, 1.4$  Hz, ArH), 6.99 (1H, dd,  $J = 7.8, 2.2$  Hz, ArH), 7.26 (1H, d,  $J = 2.4$  Hz, ArH), 7.53 (1H, s, NH), 8.16 (1H, d,  $J = 7.0$  Hz, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta$  22.70, 22.80 ( $\text{CH}_3$ ), 25.80, 28.64, 29.03, 40.89, ( $\text{CH}_2$ ), 42.13, 44.24 ( $\text{CH}$ ), 45.14 ( $\text{CH}_2$ ), 116.45, 121.42, 124.22, 124.56, 126.36, 128.36, 128.82, 130.16, 130.66, 145.16, 150.27 (ArC), 170.97, 172.22 ( $\text{C}=\text{O}$ ). HRMS: Calcd for  $\text{C}_{23}\text{H}_{26}\text{Cl}_2\text{N}_2\text{O}_3$  ( $\text{M}+\text{H}$ ) $^+$  449.1393, found ( $\text{M}+\text{H}$ ) $^+$  449.1381. Anal. Calcd for  $\text{C}_{23}\text{H}_{26}\text{Cl}_2\text{N}_2\text{O}_3$ : C 61.47, H 5.83, N 6.23. Found C 61.70, H 6.02, N 6.33.

**4-[2-(3,5-Dichloro-phenoxy)-phenylcarbamoyl]-piperidine-1-carboxylic acid tert-butyl ester, 44**,  $\text{C}_{23}\text{H}_{26}\text{Cl}_2\text{N}_2\text{O}_4$ , MW 465.38,



Using the general procedure for the formation of the amide linker the desired product was isolated as a white solid, 0.32 g, 60 % yield. m.p. 116-117 °C, LCMS:  $t_r = 5.75$  min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min),  $m/z$   $\text{M}+\text{H}$  463.46, HPLC:  $t_r = 4.30$  min (90 % acetonitrile in water at 1.0 mL/min), 96 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.43 (9H, s,  $\text{CH}_3$ ), 1.66 (2H, td,  $J = 1.2, 4.2$  Hz,  $\text{CH}_2$ ), 1.80 (2H, dd,  $J = 2.5, 12.8$  Hz,  $\text{CH}_2$ ), 2.30-2.41 (1H, m, CH), 2.74 (2H, t,  $J = 11.8$  Hz,  $\text{CH}_2\text{N}$ ), 4.07-4.12 (2H, m,  $\text{CH}_2\text{N}$ ), 6.87 (2H, d,  $J = 1.7$  Hz, ArH, ArH), 6.87-6.91 (1H, m, ArH), 7.05 (1H, td,  $J = 5.0, 6.4$  Hz, ArH), 7.11 (1H, t,  $J = 2.0$  Hz, ArH), 7.17 (1H, td,  $J = 1.5, 8.1$  Hz, ArH), 8.40 (1H, dd,  $J = 1.2, 8.1$  Hz, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta$  28.4 ( $\text{CH}_3$ ), 28.5 ( $\text{CH}_2$ ), 44.4 ( $\text{CH}$ ), 116.8, 118.7, 121.6, 124.1, 125.5 (ArCH), 129.9, 135.9, 144.1, 154.6 (ArC), 157.7, 172.6 ( $\text{CO}$ ). HRMS: Calcd for  $\text{C}_{23}\text{H}_{26}\text{Cl}_2\text{N}_2\text{O}_4$  ( $\text{M}+\text{H}$ ) $^+$  465.1353, found ( $\text{M}+\text{H}$ ) $^+$  465.1373. Anal. Calcd for  $\text{C}_{23}\text{H}_{26}\text{Cl}_2\text{N}_2\text{O}_4$ : C 59.36, H 5.63, N 6.02 %. Found C 58.90, H 5.55, N 6.49 %.

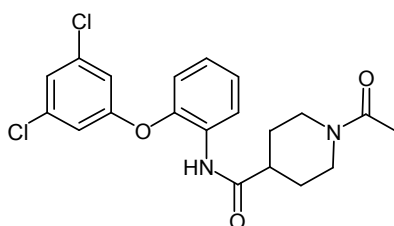
**Piperidine-4-carboxylic acid [2-(3,5-dichloro-phenoxy)-phenyl]-amide, 48b**,  $\text{C}_{19}\text{H}_{19}\text{F}_3\text{N}_2\text{O}_2$ , MW 364.37,



4-[2-(3,5-Dichloro-phenoxy)-phenylcarbamoyl]-piperidine-1-carboxylic acid tert-butyl ester (1.09 g, 2.3 mmol) was dissolved in HCl/ dioxane (4 M, 18 mL) and stirred at r.t. for 1 h. The reaction mixture was evaporated to dryness, diluted with DCM (20 mL)

and neutralised with sodium hydroxide (1 M). This was then extracted with DCM (3 x 30 mL) and the organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo* to afford the title compound as a off white solid, 183 mg, 21 % yield. m.p. 138-139 °C, R.f. 0.3 (10 % Methanol/DCM), <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.55-1.75 (3H, m, CH<sub>2</sub>, NH), 1.78-1.90 (2H, m, CH<sub>2</sub>), 2.31-2.42 (1H, m, CH), 2.65 (2H, td, *J* = 2.7, 9.6 Hz, NCH<sub>2</sub>), 3.13-3.18 (2H, m, NCH<sub>2</sub>), 6.89 (2H, d, *J* = 1.7 Hz, ArH, ArH), 6.88-6.92 (1H, m, ArH), 7.06 (1H, td, *J* = 1.5, 7.4 Hz, ArH), 7.12 (1H, t, *J* = 1.7 Hz, ArH), 7.18 (1H, td, *J* = 8.2, 1.46 Hz, ArH), 7.55 (1H, s, CONH), 8.43 (1H, dd, *J* = 8.2 Hz, ArH).

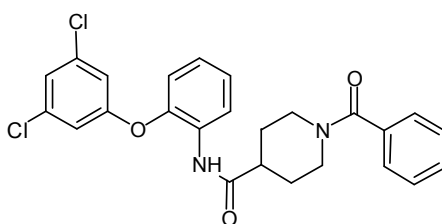
**1-Acetyl-piperidine-4-carboxylic acid [2-(3,5-dichloro-phenoxy)-phenyl]-amide, 55,**  
C<sub>20</sub>H<sub>20</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>, MW 407.3,



To a solution of piperidine-4-carboxylic acid [2-(4-trifluoromethyl-phenoxy)-phenyl]-amide (60 mg, 0.16 mmol) in DCM (5 mL) at 0 °C, was added acetyl chloride (0.02 mL, 0.32 mmol) and TEA (0.1 mL, 0.8 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 1 h. The reaction was quenched with NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL) and the organic layers were washed with 1M HCl and finally brine. The organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-5 % MeOH in EtOAc) to afford a white solid, 65 mg, 95 % yield. m.p. 112-114 °C, R.f. 0.25 (DCM), LCMS: *t*<sub>r</sub> = 1.04 min (95 % MeOH in water at 1.0 mL/min), *m/z* M+H 407.30, HPLC: *t*<sub>r</sub> = 2.15 min (90 % acetonitrile in water at 1.0 mL/min), 95 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.69-1.77 (2H, m, CH<sub>2</sub>), 1.78-1.86 (2H, m, CH<sub>2</sub>), 2.06 (3H, s, CH<sub>3</sub>), 2.37-2.47 (1H, m, CH), 2.64 (1H, td, *J* = 3.0, 13.8 Hz, NCH<sub>2</sub>), 3.07 (1H, td, *J* = 2.7, 11.9 Hz, NCH<sub>2</sub>), 3.85 (1H, d, *J* = 13.6 Hz, NCH<sub>2</sub>), 4.56 (1H, d, *J* = 13.5 Hz, NCH<sub>2</sub>), 6.87 (2H, d, *J* = 1.7 Hz, ArH, ArH), 6.90 (1H, dd, *J* = 1.2, 8.2 Hz, ArH), 7.06 (1H, td, *J* = 1.5, 7.7 Hz, ArH), 7.11 (1H, t, *J* = 2.0 Hz, ArH), 7.18 (td, *J* = 1.2, 7.9 Hz, ArH), 7.64 (1H, s, NH), 8.36 (1H, dd, *J* = 1.0, 8.2 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 21.5 (CH<sub>3</sub>), 28.5, 28.8, 40.8 (CH<sub>2</sub>), 44.1 (CH), 45.7 (CH<sub>2</sub>), 116.8, 118.8, 121.7, 124.1, 124.7, 125.6 (ArCH), 129.8, 136.0, 144.2, 157.8 (ArC), 168.9, 172.3 (CO). Anal. Calcd for C<sub>20</sub>H<sub>20</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>: C 58.98, H 4.95, N 6.88 %. Found: C 58.6, H 4.91, N 6.61 %, HRMS: Calcd for C<sub>20</sub>H<sub>20</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 407.0924, found (M+H)<sup>+</sup> 407.0929.

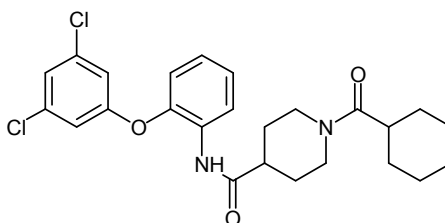


**1-Benzoyl-piperidine-4-carboxylic acid [2-(3,5-dichloro-phenoxy)-phenyl]-amide, 56**, C<sub>25</sub>H<sub>22</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>, MW 469.37,



To a solution of piperidine-4-carboxylic acid [2-(3,5-dichloro-phenoxy)-phenyl]-amide (60 mg, 0.16 mmol) in DCM (5 mL) at 0 °C, was added benzoyl chloride (0.02 mL, 0.16 mmol) and TEA (0.1 mL, 0.8 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 1 h. The reaction was quenched with sat. NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL), washed with 1M HCl and finally brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>) and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-5 % MeOH in EOAc) to afford the desired compound as a white solid, 50 mg, 64 % yield. m.p. 61-63 °C, R.f. 0.3 (DCM), LCMS: *t*<sub>r</sub> = 1.3 min (95 % MeOH in water at 1.0 mL/min), *m/z* M+H 469.37, HPLC: *t*<sub>r</sub> = 3.6 min (90 % acetonitrile in water at 1.0 mL/min), 98 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 1.77-1.92 (4H, m, 2CH<sub>2</sub>), 2.46-2.55 (1H, m, CH), 2.86 (1H, s, CH<sub>2</sub>), 3.01 (1H, s, CH<sub>2</sub>), 3.77 (1H, s, NCH<sub>2</sub>), 4.65 (1H, s, NCH<sub>2</sub>), 6.88 (2H, d, *J* = 1.6 Hz, ArH), 6.91 (1H, dd, *J* = 1.2, 8.0 Hz, ArH), 7.08 (1H, td, *J* = 1.2, 7.6 Hz, ArH), 7.12 (1H, t, *J* = 1.6 Hz, ArH), 7.20 (1H, td, *J* = 1.2, 8.0 Hz, ArH), 7.35-7.42 (5H, m, ArH), 7.71 (1H, s, NH), 8.38 (1H, dd, *J* = 0.8, 8.0 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 14.4, 22.9, 29.1, 31.9 (CH<sub>2</sub>), 44.5 (CH), 117.1, 119.1, 122.0, 124.4, 124.9, 125.9, 127.2, 128.8, 130.0 (ArCH), 130.1, 136.1, 136.3, 144.5, 158.0 (ArC), 170.8, 172.4 (CO). HRMS: Calcd for C<sub>25</sub>H<sub>22</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 469.1080, found (M+H)<sup>+</sup> 469.1082. Anal. Calcd for C<sub>25</sub>H<sub>22</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>: C 63.97, H 4.72, N 5.97 %. Found: C 63.4, H 4.7, N 5.95 %.

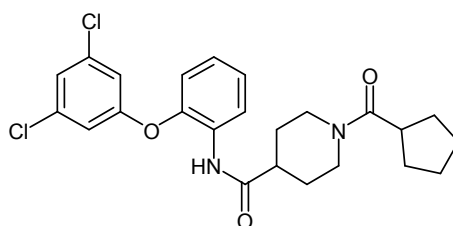
**1-Cyclohexanecarbonyl-piperidine-4-carboxylic acid [2-(3,5-dichloro-phenoxy)-phenyl]-amide, 64**, C<sub>25</sub>H<sub>28</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>, MW 475.41,



To a solution of piperidine-4-carboxylic acid [2-(3,5-dichloro-phenoxy)-phenyl]-amide (0.07 g, 0.18 mmol) in DCM (4 mL), at 0 °C, was added TEA (0.15 mL) and cyclohexane carbonyl chloride (0.05 mL, 0.36 mmol). The reaction mixture was allowed to warm to room temperature, and stirred for 30 min. The reaction was quenched with sat. NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL), washed with 1M HCl (20 mL) and brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>) and

evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % EtOAc in hexane) to afford the title compound as a white waxy solid, 71 mg, 83 % yield. m.p. 64-67 °C, R.f. 0.6 (EtOAc), LCMS:  $t_r$  = 2.0 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min),  $m/z$  M+H 475.29, HPLC:  $t_r$  = 3.16 min (90 % acetonitrile in water at 1.0 mL/min), >99%,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.21-1.27 (4H, m, 2CH<sub>2</sub>), 1.44-1.93 (10H, m, 5CH<sub>2</sub>), 2.39-2.51 (2H, m, 2CH), 2.61 (1H, t,  $J$  = 11.6 Hz, CH<sub>2</sub>), 3.04 (1H, t,  $J$  = 11.9 Hz, CH<sub>2</sub>), 3.96 (1H, d,  $J$  = 13.6 Hz, CH<sub>2</sub>), 4.61 (1H, d,  $J$  = 13.4 Hz, CH<sub>2</sub>), 6.88 (2H, d,  $J$  = 2.0 Hz, ArH), 6.90 (1H, dd,  $J$  = 8.15, 1.2 Hz, ArH), 7.07 (1H, td,  $J$  = 7.9, 1.7 Hz, ArH), 7.12 (1H, t,  $J$  = 2.0 Hz, ArH), 7.18 (1H, td,  $J$  = 1.2, 7.6 Hz, ArH), 7.61 (1H, s, NH), 8.39 (1H, dd,  $J$  = 1.5, 8.2 Hz, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz):  $\delta$  25.95, 28.72, 29.36, 29.65 (CH<sub>2</sub>), 40.56 (CH), 41.05 (CH<sub>2</sub>), 44.39 (CH), 44.72 (CH<sub>2</sub>), 116.90, 118.91, 121.78, 124.18, 124.75, 125.62 (ArCH), 129.90, 136.07, 144.24, 157.83 (ArC), 172.37, 174.63 (C=O), HRMS: Calcd for C<sub>25</sub>H<sub>28</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 475.1550, found (M+H)<sup>+</sup> 475.1544.

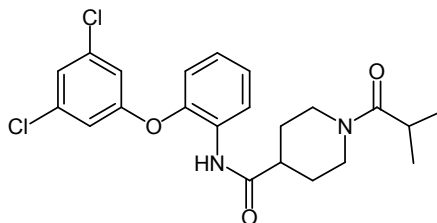
**1-Cyclopentanecarbonyl-piperidine-4-carboxylic acid [2-(3,5-dichloro-phenoxy)-phenyl]-amide, 58**, C<sub>24</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>, MW 461.38,



To a cooled solution of piperidine-4-carboxylic acid [2-(3,5-dichloro-phenoxy)-phenyl]-amide (0.07 g, 0.18 mmol) in DCM (4 mL) at 0 °C, was added TEA (0.15 mL) and cyclopentane carbonyl chloride (0.044 mL, 0.36 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction was quenched with sat. NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL), washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>) and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to afford the title compound as a white waxy solid, 52 mg, 63 % yield. m.p. 154-155 °C, R.f. 0.62 (EtOAc), LCMS:  $t_r$  = 1.5 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min),  $m/z$  M+H 461.38, HPLC:  $t_r$  = 3.04 min (90 % acetonitrile in water at 1.0 mL/min), 99 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.53-1.92 (12H, m, 6CH<sub>2</sub>), 2.41-2.52 (1H, m, CH), 2.66 (1H, t,  $J$  = 11.9 Hz, CH<sub>2</sub>), 2.81-2.92 (1H, m, CH), 3.06 (1H, t,  $J$  = 11.1 Hz, CH<sub>2</sub>), 4.00 (1H, d,  $J$  = 13.9 Hz, CH<sub>2</sub>), 4.62 (1H, d,  $J$  = 12.8 Hz, CH<sub>2</sub>), 6.88-6.92 (3H, m, ArH), 7.07 (1H, td,  $J$  = 7.4, 1.5 Hz, ArH), 7.13 (1H, t,  $J$  = 1.7 Hz, ArH), 7.19 (1H, td,  $J$  = 7.9, 1.5 Hz, ArH), 7.58 (1H, s, NH), 8.40 (1H, d,  $J$  = 7.9 Hz, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta$  26.12, 28.70, 29.17, 30.09, 30.34 (CH<sub>2</sub>), 41.19 (CH), 41.26 (CH<sub>2</sub>), 44.44 (CH),

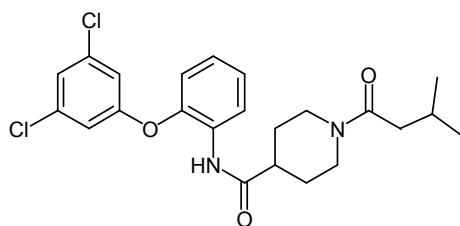
44.84 (CH<sub>2</sub>), 116.84, 118.90, 121.73, 124.19, 124.73, 125.64 (ArCH), 129.91, 136.09, 144.19, 157.83 (ArC), 172.36, 174.53 (C=O). HRMS: Calcd for C<sub>24</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 461.1393, found (M+H)<sup>+</sup> 461.1394.

**1-Isobutyryl-piperidine-4-carboxylic acid [2-(3,5-dichloro-phenoxy)-phenyl]-amide, 59**, C<sub>22</sub>H<sub>24</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>, MW 435.34,



To a solution of piperidine-4-carboxylic acid [2-(3,5-dichloro-phenoxy)-phenyl]-amide (0.07 g, 0.18 mmol) in DCM (4 mL) at 0 °C was added TEA (0.15 mL) and isobutyryl chloride (0.038 mL, 0.36 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction was quenched with sat. NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL) and washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>) and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to afford a white wax, 77 mg, 97 % yield. R.f. 0.7 (EtOAc), LCMS: *t*<sub>r</sub> = 1.9 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min), *m/z* M+H 435.38, HPLC: *t*<sub>r</sub> = 2.7 min (90 % acetonitrile in water at 0.8 mL/min), 98 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.09 (6H, t, *J* = 5.5 Hz, 2CH<sub>3</sub>), 1.62-1.76 (2H, m, CH<sub>2</sub>), 1.78-1.92 (2H, m, CH<sub>2</sub>), 2.41-2.52 (1H, m, CH<sub>2</sub>), 2.63 (1H, t, *J* = 11.9 Hz, CH<sub>2</sub>), 2.72-2.85 (1H, m, CH), 3.06 (1H, t, *J* = 11.4 Hz, CH<sub>2</sub>), 3.96 (1H, d, *J* = 13.6 Hz, CH<sub>2</sub>), 4.60 (1H, d, *J* = 13.1 Hz, CH<sub>2</sub>), 6.86-6.92 (3H, m, ArH), 7.06 (1H, td, *J* = 1.8, 8.2 Hz, ArH), 7.11 (1H, t, *J* = 1.8 Hz, ArH), 7.18 (1H, td, *J* = 1.6, 7.7 Hz, ArH), 7.64 (1H, s, NH), 8.37 (1H, dd, *J* = 1.2, 8.2 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): δ 19.41, 19.67 (CH<sub>3</sub>), 28.70, 29.21 (CH<sub>2</sub>), 30.19 (CH), 41.13 (CH<sub>2</sub>), 44.31 (CH), 44.74 (CH<sub>2</sub>), 116.82, 118.95, 121.86, 124.14, 124.78, 125.62 (ArCH), 129.91, 136.06, 144.28, 157.85 (ArC), 172.38, 175.41 (CO). HRMS: Calcd for C<sub>22</sub>H<sub>24</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 435.1237, found (M+H)<sup>+</sup> 435.1240.

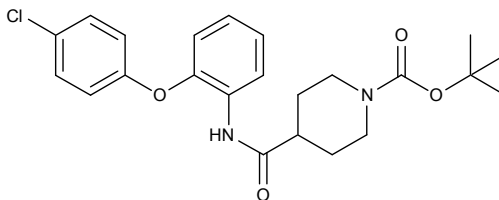
**1-(3-Methyl-butyryl)-piperidine-4-carboxylic acid [2-(3,5-dichloro-phenoxy)-phenyl]-amide, 60**, C<sub>22</sub>H<sub>24</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>, MW 448.1,



To a solution of piperidine-4-carboxylic acid [2-(3,5-dichloro-phenoxy)-phenyl]-amide (0.07 g, 0.18 mmol) in DCM (4 mL), at 0 °C was added TEA (0.15 mL) and isovaleryl

chloride (0.044 mL, 0.36 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction was quenched with sat. NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL) and washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>) and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to afford the desired compound as a white waxy solid, 52 mg, 72% yield. mp. 119-121 °C, R.f. 0.65 (EtOAc), LCMS: *t*<sub>r</sub> = 1.8 min (50 % to 95% MeOH in Water at 0.5 mL/min to 1.0 mL/min over 5 min), *m/z* M+H 449.4, HPLC: *t*<sub>r</sub> = 2.9 min (90 % acetonitrile in water at 0.8 mL/min), 99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 0.94 (6H, d, *J* = 6.4 Hz, 2CH<sub>3</sub>), 1.56-1.77 (2H, m, CH<sub>2</sub>), 1.84-1.91 (2H, m, CH<sub>2</sub>), 2.02-2.13 (1H, m, CH), 2.17-2.21 (2H, m, CH<sub>2</sub>), 2.40-2.51 (1H, m, CH), 2.64 (1H, t, *J* = 10.9 Hz, CH<sub>2</sub>), 3.05 (1H, t, *J* = 11.9 Hz, CH<sub>2</sub>), 3.92 (1H, d, *J* = 13.6 Hz, CH<sub>2</sub>), 4.61 (1H, d, *J* = 13.4 Hz, CH<sub>2</sub>), 6.87 (2H, d, *J* = 1.7 Hz, ArH), 6.87-6.91 (1H, m, ArH), 7.06 (1H, td, *J* = 7.9 Hz, ArH), 7.12 (1H, t, *J* = 1.7 Hz, ArH), 7.18 (1H, td, *J* = 1.5 Hz, ArH), 7.61 (1H, s, NH), 8.39 (1H, dd, *J* = 1.2, 8.2 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): δ 22.80, 22.92 (CH<sub>3</sub>), 25.89 (CH), 28.72, 29.09, 40.95, 42.21 (CH<sub>2</sub>), 44.26 (CH), 45.19 (CH<sub>2</sub>), 116.85, 118.91, 121.78, 124.18, 124.76, 125.63 (ArCH), 129.89, 136.07, 144.24, 157.83 (ArC), 171.04, 172.34 (C=O). HRMS: Calcd for C<sub>22</sub>H<sub>24</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 449.1404, found (M+H)<sup>+</sup> 449.1394.

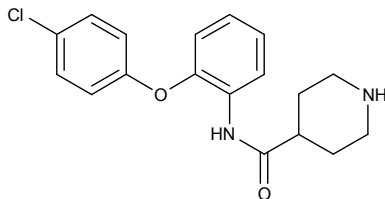
**4-[2-(2,4-Dichlorophenoxy)phenylcarbamoyl]piperidine-1-carboxylic acid *tert*-butyl ester, 45**, C<sub>23</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>4</sub>, MW 465.37,



Using the general procedure for the formation of the amide linker the desired product was obtained as a white solid, 754 mg, 84 % yield. R.f. 0.31 (Hexane:EtOAc 7:3), mp 120-121 °C, LC/MS *t*<sub>r</sub> = 1.39 min, *m/z* M-H 464.96, <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) δ 1.44 (9H, s, 3CH<sub>3</sub>), 1.65-1.76 (2H, m, CH<sub>2</sub>), 1.84-1.86 (2H, br d, CH<sub>2</sub>), 2.40 (1H, tt, CH), 2.77 (2 H, br t, CH<sub>2</sub>), 4.14 (2H, br d, CH<sub>2</sub>), 6.71 (1H, dd, *J* = 8.2, 1.7 Hz, ArH), 6.94 (1H, d, *J* = 8.9 Hz, ArH), 7.01 (1H, td, *J* = 8.2, 1.5, ArH), 7.12 (1H, td, *J* = 8.2, 1.5 Hz, ArH), 7.21 (1H, dd, *J* = 8.9, 2.5 Hz, ArH), 7.48 (1H, d, *J* = 2.5 Hz, ArH), 7.76 (1H, br s, NH) and 8.40 (1H, dd, *J* = 7.9, 1.5 Hz, ArH).

**Piperidine-4-carboxylic acid [2-(4-chlorophenoxy)phenyl]amide, 48c,**

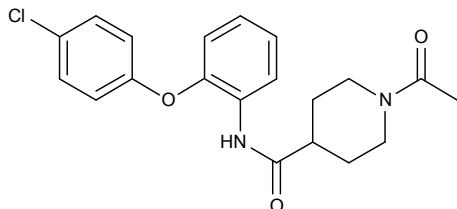
C<sub>18</sub>H<sub>19</sub>ClN<sub>2</sub>O<sub>2</sub>, MW 330.81,



A solution of 4-[2-(4-chlorophenoxy)phenylcarbamoyl]piperidine-1-carboxylic acid *tert*-butyl ester (410 mg, 0.95 mmol) in 4M HCl in dioxane (2 mL) was stirred at room temperature for 2 h. The resulting solution was concentrated under vacuum and the residue was dissolved in DCM (20 mL), washed with 1M NaOH (1 x 20 mL), water (20 mL) and brine (20 mL). The organic layer was dried (MgSO<sub>4</sub>), filtered and evaporated to give the title compound as a white solid, 233 mg, 74 % yield. R.f: 0.11 (DCM: methanol, 4:1 plus 3 drops of TEA), m.p. 138-140 °C, <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) δ 1.55-1.70 (2H, m, CH<sub>2</sub>), 1.80-1.85 (3H, m, CH<sub>2</sub> + NH), 2.35 (1H, tt, CH), 2.62 (2H, br t, CH<sub>2</sub>), 3.13 (2H, br d, CH<sub>2</sub>), 6.80 (1H, dd, ArH), 6.92 (2H, AA'BB', ArH), 6.98 (1H, td, ArH), 7.11 (1H, td, ArH), 7.29 (1H, ArH), 7.71 (1H, br s, NHCO) and 8.41 (1H, br d, *J* = 7.9 Hz, ArH).

**1-Acetylpiperidine-4-carboxylic acid [2-(4-chlorophenoxy)phenyl]amide, 61,**

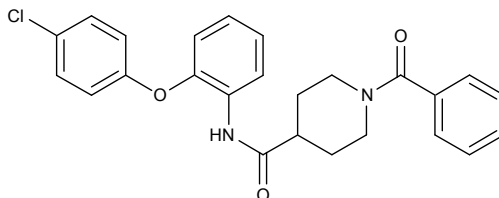
C<sub>20</sub>H<sub>21</sub>ClN<sub>2</sub>O<sub>3</sub>, MW 372.85,



To an ice cooled solution of piperidine-4-carboxylic acid [2-(4-chlorophenoxy)phenyl]amide (100 mg, 0.3 mmol) in dry DCM (6 mL) were added TEA (2.1 mL, 15 mmol) and acetyl chloride (0.043 mL, 0.6 mmol). The reaction mixture was stirred at room temperature for 1.5 h and quenched with saturated NaHCO<sub>3</sub>. The resulting solution was extracted with DCM (3 x 20 mL) and the combined organic layers were washed with water (20 mL), 1M HCl (20 mL) and brine (20 mL), dried (MgSO<sub>4</sub>), filtered and evaporated. Flash chromatography of the crude product (0-50% EtOAc in hexane) gave the title compound as a white solid, 37 mg, 33 % yield. R.f: 0.15 (EtOAc), m.p. 195-196 °C, LC/MS (APCI) *t*<sub>r</sub> = 1.04 min, *m/z* 373.36 (M+H, 100), HPLC *t*<sub>r</sub> = 2.2 min, >99 %, <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) δ 1.64-1.77 (2H, m, CH<sub>2</sub>), 1.79-1.94 (2H, m, CH<sub>2</sub>), 2.08 (3H, s CH<sub>3</sub>), 2.46 (1H, tt, CH), 2.66 (1H, m, ½CH<sub>2</sub>), 3.09 (1H, m, ½CH<sub>2</sub>), 3.86 (1H, m, ½CH<sub>2</sub>), 4.55 (1H, m, ½CH<sub>2</sub>), 6.82 (1H, dd, *J* = 8.2, 1.5 Hz, ArH), 6.93-6.95 (2H, m, ArH), 7.01 (1H, td, ArH), 7.12 (1 H, td, ArH), 7.28-7.32 (2H, m, ArH), 7.69 (1H, br s, NH) and 8.38 (1H, dd, *J* = 8.2, 1.2 Hz, ArH). <sup>13</sup>C NMR

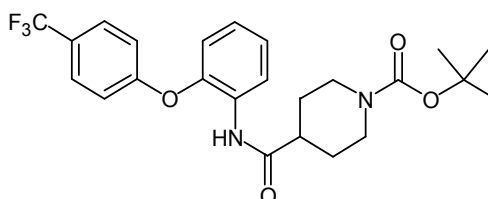
(400 MHz, CDCl<sub>3</sub>)  $\delta$  21.53 (CH<sub>3</sub>), 28.47, 28.89, 40.81 (CH<sub>2</sub>), 44.15 (CH), 45.70 (CH<sub>2</sub>), 117.77, 119.87, 121.31, 124.44, 124.54, 129.19, 129.45, 130.07, 154.44, 154.94, 168.95 (ArC), 172.20 (C=O). HRMS: Calcd for C<sub>20</sub>H<sub>21</sub>ClN<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 373.1313, found (M+H)<sup>+</sup> 373.1314.

**1-Benzoylpiperidine-4-carboxylic acid [2-(4-chlorophenoxy) phenyl]amide, 62**, C<sub>25</sub>H<sub>23</sub>ClN<sub>2</sub>O<sub>3</sub>, MW 434.91,



To an ice cooled solution of piperidine-4-carboxylic acid [2-(4-chlorophenoxy)phenyl]amide (73 mg, 0.22 mmol) in dry DCM (6 mL) were added pyridine (0.036 mL, 0.44 mmol) and benzoyl chloride (0.038 mL, 0.33 mmol). The reaction mixture was stirred at room temperature for 1 h and quenched with saturated NaHCO<sub>3</sub>. The resulting solution was extracted with DCM (2 x 20 mL) and the combined organic layers were washed with water (10 mL), 1M HCl (20 mL) and brine (20 mL), dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. Flash chromatography of the crude product (0-100 % EtOAc in hexane) gave the desired compound as a white solid 80 mg, 83 % yield. R.f: 0.48 (EtOAc), mp 158-160 °C, LC/MS (APCI)  $t_r$  = 1.05 min,  $m/z$  435.38 (M+H, 100), HPLC  $t_r$  = 2.6 min, 98 %. <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>)  $\delta$  1.82-2.03 (4H, m, 2CH<sub>2</sub>), 2.51 (1H, tt, CH), 2.98 (2H, m, CH<sub>2</sub>), 3.85 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 4.71 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 6.82 (1H, dd,  $J$  = 8.2, 1.5 Hz, ArH), 6.93-6.95 (2H, m, ArH), 7.02 (1H, td, ArH), 7.13 (1H, td, ArH), 7.29-7.33 (1H, m, ArH), 7.39 (5H, s, ArH), 7.70 (1H, br s, NH) and 8.39 (1H, dd,  $J$  = 8.2, 1.5 Hz, ArH). Anal. Calcd. for C<sub>25</sub>H<sub>23</sub>ClN<sub>2</sub>O<sub>3</sub>: C 69.04, H 5.33, N 6.44. Found: C 68.9, H 5.33, N 6.37%.

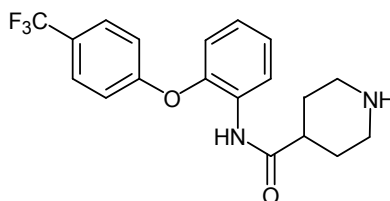
**4-[2-(4-Trifluoromethyl-phenoxy)-phenylcarbamoyl]-piperidine-1-carboxylic acid tert-butyl ester, 46**, C<sub>24</sub>H<sub>27</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub>, MW 464.49



Using the general procedure for the formation of the amide linker the desired compound was obtained as a white solid, 1.28 g, 72 % yield. m.p. 48-51 °C, R.f 0.44 (DCM: Hexane, 1:1), LCMS:  $t_r$  = 5.0 min (50 % MeOH in water at 0.5 mL/min),  $m/z$  M+H 463.27, HPLC:  $t_r$  = 2.6 min (90 % acetonitrile in water at 1.0 mL/min), 99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  1.44 (9H, s, CH<sub>3</sub>), 1.54-1.70 (2H, m, CH<sub>2</sub>), 1.75-1.81 (2H, m,

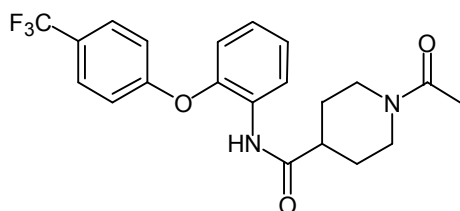
CH<sub>2</sub>), 2.31-2.38 (1H, m, CH), 2.73 (2H, t,  $J$  = 11.8 Hz, CH<sub>2</sub>), 4.13 (2H, s, CH<sub>2</sub>), 6.91 (1H, dd,  $J$  = 1.5, 8.2 Hz, ArH), 7.06 (1H, td,  $J$  = 1.8, 8.2 Hz, ArH), 7.06 (2H, d,  $J$  = 8.4 Hz, ArH), 7.18 (1H, td,  $J$  = 1.8, 9.2 Hz, ArH), 7.56 (1H, s, NH), 7.60 (2H, d,  $J$  = 8.4 Hz, ArH), 8.4 (1H, dd,  $J$  = 8.2 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta$  28.38 (CH<sub>3</sub>), 28.46 (CH<sub>2</sub>), 117.8, 118.9, 121.7, 124.5, 125.4, 127.4, 127.5 (ArCH), 129.9, 144.4 (ArC), 154.6 (CF<sub>3</sub>), 159.4, 172.6 (CO). Anal. Calcd for C<sub>24</sub>H<sub>27</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub>: C 62.06, H 5.86, N 6.03 %. Found C 62.3, H 6.39, N 5.91 %.

**Piperidine-4-carboxylic acid [2-(4-trifluoromethyl-phenoxy)-phenyl]-amide, 48d**, C<sub>19</sub>H<sub>19</sub>F<sub>3</sub>N<sub>2</sub>O<sub>2</sub> MW 364.37,



Using the general procedure for the removal of the *N*-Boc protecting group the desired compound was obtained as a cream oil, 0.14 g, 89 % yield. R.f: 0.47 (DCM), <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 270 MHz):  $\delta$  1.56-1.72 (2H, m, CH<sub>2</sub>), 1.73-1.83 (2H, m, CH<sub>2</sub>), 2.24-2.41 (1H, m, CH), 2.64 (2H, td,  $J$  = 12.4, 2.7 Hz, NCH<sub>2</sub>), 3.11-3.16 (2H, m, NCH<sub>2</sub>), 6.97 (1H, d,  $J$  = 6.4 Hz, ArH), 7.03-7.08 (3H, m, ArH), 7.17 (1H, t,  $J$  = 7.2 Hz, ArH), 7.58, (1H, d,  $J$  = 8.6 Hz, ArH), 8.39 (1H, d,  $J$  = 7.9 Hz, ArH).

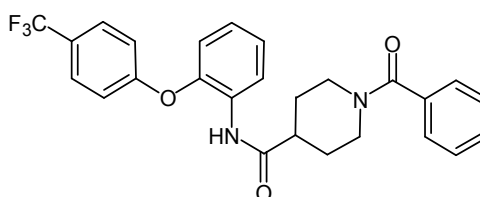
**1-Acetyl-piperidine-4-carboxylic acid [2-(4-trifluoromethyl-phenoxy)-phenyl]-amide, 63**, C<sub>21</sub>H<sub>21</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub> MW 406.40,



To a solution of piperidine-4-carboxylic acid [2-(4-trifluoromethyl-phenoxy)-phenyl]-amide (70 mg, 0.2 mmol) in DCM (5 mL) at 0 °C, was added acetyl chloride (0.03 mL, 0.4 mmol) and TEA (0.12 mL). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction was quenched with sat. NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL) and washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-10% methanol/ethyl acetate), to afford a white solid, 38 mg, 49 % yield. m.p. 164-165 °C, R.f. 0.4 (5 % MeOH/ EtOAc), LCMS:  $t_r$  = 1.00 min (95 % MeOH and 5 % water at 1.0 mL/min),  $m/z$  M+H 405.22, HPLC:  $t_r$  = 2.2 min (90 % acetonitrile in water at 1.0 mL/min), 95 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  1.56-1.75 (2H, m, CH<sub>2</sub>), 1.83 (2H, s, CH<sub>2</sub>), 2.09 (3H, s,

CH<sub>3</sub>), 2.37-2.48 (1H, m, CH), 2.64 (1H, td,  $J = 14.1, 2.7$  Hz, NCH<sub>2</sub>), 3.07 (1H, td,  $J = 14.6, 2.9$  Hz, NCH<sub>2</sub>), 3.83 (1H, d,  $J = 13.9$  Hz, NCH<sub>2</sub>), 4.57 (1H, d,  $J = 13.4$  Hz, NCH<sub>2</sub>), 6.90 (1H, dd,  $J = 8.2, 1.2$  Hz, ArH), 7.04-7.08 (2H, m, ArH), 7.09-7.10 (1H, m, ArH), 7.19 (1H, td,  $J = 1.5, 9.2$  Hz, ArH), 7.60 (2H, d,  $J = 8.9$  Hz, ArH), 7.60 (1H, s, NH), 8.39 (1H, d,  $J = 7.9$  Hz, ArH). <sup>13</sup>C NMR: (CDCl<sub>3</sub>, 101 MHz):  $\delta$  21.4 (CH<sub>3</sub>), 28.3, 28.8, 40.7 (CH<sub>2</sub>), 44.0 (CH), 45.6 (CH<sub>2</sub>), 117.8, 118.9, 121.7, 124.7, 125.3, 127.4, 127.4, 127.5 (ArCH), 127.5, 129.8, 144.5, 159.3 (ArC), 168.9, 172.1 (CO). HRMS: Calcd for C<sub>21</sub>H<sub>21</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 407.1577, found (M+H)<sup>+</sup> 407.1574. Anal. Calcd for C<sub>21</sub>H<sub>21</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub>: C 62.06, H 5.21, N 6.89 %. Found: C 61.3, H 5.22, N 6.65 %.

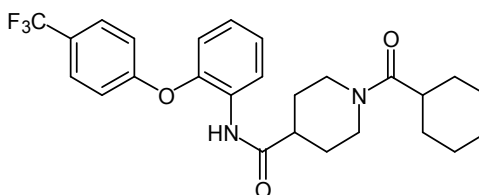
**1-Benzoyl-piperidine-4-carboxylic acid [2-(4-trifluoromethyl-phenoxy)-phenyl]-amide, 64**, C<sub>26</sub>H<sub>23</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub>, MW 468.47



To a solution of piperidine-4-carboxylic acid [2-(4-trifluoromethyl-phenoxy)-phenyl]-amide (70 mg, 0.2 mmol) in DCM (5 mL) at 0 °C was added benzoyl chloride (0.05 mL, 0.4 mmol) and TEA (0.1 mL). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction was quenched with NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL) and washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-5% methanol in ethyl acetate), to afford the desired compound as a white solid, 57 mg, 63 % yield. m.p. 69-72 °C, R.f. 0.45 (5 % MeOH/ EtOAc), LCMS:  $t_r$ =1.15 min (95 % MeOH and 5% water at 1.0 mL/min), M+H 469.44, HPLC:  $t_r$ = 2.13 min (90 % acetonitrile in water at 1.0 mL/min), 95 %. <sup>1</sup>H NMR: (CDCl<sub>3</sub>, 270 MHz):  $\delta$  1.78-2.02 (4H, m, CH<sub>2</sub>), 2.42-2.52 (1H, m, CH), 2.96-3.06 (2H, m, CH<sub>2</sub>), 3.81-3.91 (1H, m, CH<sub>2</sub>), 4.73 (1H, s, CH<sub>2</sub>), 6.85-6.96 (1H, m, ArH), 7.00-7.08 (2H, m, ArH), 7.13-7.21 (1H, m, ArH), 7.23-7.27 (1H, m, ArH), 7.38 (5H, s, ArH), 7.58-7.61 (3H, m, CH<sub>2</sub>, NH), 8.40 (1H, d,  $J = 7.9$  Hz, ArH). <sup>13</sup>C NMR: (CDCl<sub>3</sub>, 101 MHz):  $\delta$  28.5, 28.7, 41.4 (CH<sub>2</sub>), 44.1 (CH), 46.9 (CH<sub>2</sub>), 117.8, 118.9, 121.8, 124.7, 125.3, 126.8, 127.4, 127.4, 128.5, 129.7 (ArCH), 129.8, 135.7, 144.5, 159.3 (ArC), 170.4, 172.2 (CO). <sup>19</sup>F NMR (CDCl<sub>3</sub>, 376 MHz):  $\delta$  -61.84 (s, CF<sub>3</sub>). HRMS: Calcd for C<sub>26</sub>H<sub>23</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 469.1734, found (M+H)<sup>+</sup> 469.1731. Anal. Calcd for C<sub>26</sub>H<sub>23</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub>: C 66.66, H 4.95, N 5.98 %. Found: C 66.4, H 5.03, N 5.72 %.

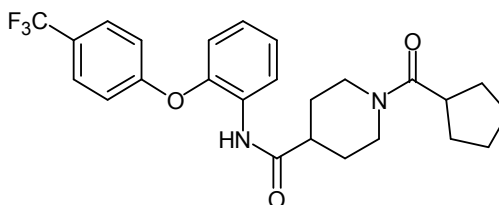


**1-Cyclohexanecarbonyl-piperidine-4-carboxylic acid [2-(4-trifluoromethyl-phenoxy)-phenyl]-amide, 65**, C<sub>26</sub>H<sub>29</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub>, MW 474.52



To a solution of piperidine-4-carboxylic acid [2-(4-trifluoromethyl-phenoxy)-phenyl]-amide (0.1 g, 0.3 mmol) in DCM (5 mL) at 0 °C was added TEA (0.2 mL) and cyclohexane carbonyl chloride (0.08 mL, 0.54 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction was quenched with sat. NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL) and washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to afford the title compound as a white waxy solid, 128 mg, 98 % yield. m.p. 78-80 °C, R.f. 0.6 (EtOAc), LCMS: *t*<sub>r</sub> = 4.7 min (50 % MeOH in water at 0.5 mL/min), *m/z* M+H 475.42, HPLC: *t*<sub>r</sub> = 2.6 min (90 % acetonitrile in water at 1.0 mL/min), 99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 1.48-1.88 (14H, m, 7CH<sub>2</sub>), 2.39-2.48 (2H, m, 2CH), 2.60 (1H, t, *J* = 11.2 Hz, CH<sub>2</sub>), 3.02 (1H, t, *J* = 12.0 Hz, CH<sub>2</sub>), 3.93 (1H, d, *J* = 13.2 Hz, CH<sub>2</sub>), 4.59 (1H, d, *J* = 13.2 Hz, CH<sub>2</sub>), 6.91 (1H, dd, *J* = 8.0, 1.2 Hz, ArH), 7.07 (2H, d, *J* = 8.8 Hz, ArH), 7.07 (1H, m, ArH), 7.18 (1H, td, *J* = 1.2, 8.0 Hz, ArH), 7.60 (2H, d, *J* = 9.2 Hz, ArH), 7.62 (1H, s, NH), 8.38 (1H, dd, *J* = 1.2, 8.0 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 25.79, 28.51, 29.00, 29.23, 29.50 (CH<sub>2</sub>), 40.42 (CH), 40.88 (CH<sub>2</sub>), 44.25 (CH), 44.57 (CH<sub>2</sub>), 117.79, 118.94, 121.75, 122.56, 124.62, 125.31, 127.37, 127.40, 127.43, 127.47, 129.86, 144.44 (ArC), 159.34 (CF<sub>3</sub>), 172.23, 174.49 (C=O). HRMS: Calcd for C<sub>26</sub>H<sub>29</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 475.2203, found (M+H)<sup>+</sup> 475.2198.

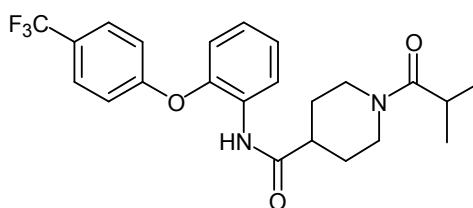
**1-Cyclopentanecarbonyl-piperidine-4-carboxylic acid [2-(4-trifluoromethyl-phenoxy)-phenyl]-amide, 66**, C<sub>25</sub>H<sub>27</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub>, MW 460.49,



To a solution of piperidine-4-carboxylic acid [2-(4-trifluoromethyl-phenoxy)-phenyl]-amide (0.1 g, 0.3 mmol) in DCM (5 mL) at 0 °C was added TEA (0.2 mL) and cyclopentane carbonyl chloride (0.07 mL, 0.54 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction was quenched with sat. NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL) and washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried

(MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to afford the title compound as a white waxy solid, 105 mg, 83 % yield. m.p. 94-97 °C, R.f. 0.65 (EtOAc), LCMS: *t*<sub>r</sub> = 4.86 min (50 % MeOH in water at 0.5 mL/min), *m/z* M+H 461.44, HPLC: *t*<sub>r</sub> = 2.43 min (90 % acetonitrile in water at 1.0 mL/min), 99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 1.48-1.86 (12H, m, 6CH<sub>2</sub>), 2.40-2.48 (1H, m, CH), 2.63 (1H, td, *J* = 13.6, 2.8 Hz, CH<sub>2</sub>), 2.81-2.89 (1H, m, CH), 3.03 (1H, td, *J* = 14.4, 2.4 Hz, CH<sub>2</sub>), 3.98 (1H, d, *J* = 13.2 Hz, CH<sub>2</sub>), 4.59 (1H, d, *J* = 13.2 Hz, CH<sub>2</sub>), 6.91 (1H, dd, *J* = 1.2, 7.6 Hz, ArH), 7.05 (2H, d, *J* = 8.0 Hz, ArH), 7.08 (1H, td, *J* = 1.6 Hz, ArH), 7.18 (1H, td, *J* = 1.2, 8.8 Hz, ArH), 7.59 (2H, d, *J* = 8.4 Hz, ArH), 7.64 (1H, s, NH), 8.37 (1H, dd, *J* = 1.2, 7.2 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 25.95, 28.47, 29.03, 29.92 (CH<sub>2</sub>), 41.02 (CH), 41.09 (CH<sub>2</sub>), 44.22 (CH), 44.69 (CH<sub>2</sub>), 117.76, 118.94, 121.76, 124.61, 125.29, 127.33, 127.37, 127.41, 127.45, 129.85, 144.44 (ArC), 159.33 (CF<sub>3</sub>), 172.26, 174.40 (C=O). HRMS: Calcd for C<sub>25</sub>H<sub>27</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 461.2047, found (M+H)<sup>+</sup> 461.2035.

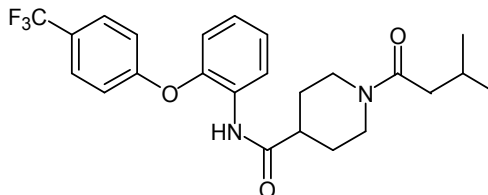
**1-Isobutyryl-piperidine-4-carboxylic acid [2-(4-trifluoromethyl-phenoxy)-phenyl]-amide, 67**, C<sub>23</sub>H<sub>25</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub>, MW 434.45,



To a solution of piperidine-4-carboxylic acid [2-(4-trifluoromethyl-phenoxy)-phenyl]-amide (0.1 g, 0.3 mmol) in DCM (5 mL) at 0 °C was added TEA (0.2 mL) and isobutyryl carbonyl chloride (0.06 mL, 0.5 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction was quenched with sat. NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL) and washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to afford the title compound as a white waxy solid, 98 mg, 82 % yield. m.p. 70-72 °C, R.f. 0.60 (EtOAc), LCMS: *t*<sub>r</sub> = 4.28 min (50 % MeOH in water at 0.5 mL/min), *m/z* M+H 435.44, HPLC: *t*<sub>r</sub> = 2.23 min (90 % acetonitrile in water at 1.0 mL/min), 96 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.08 (6H, t, *J* = 6.0 Hz, CH<sub>3</sub>), 1.57-1.70 (2H, m, CH<sub>2</sub>), 1.80-1.86 (2H, m, CH<sub>2</sub>), 2.41-2.48 (1H, m, CH), 2.60 (1H, t, *J* = 12.8 Hz, CH<sub>2</sub>), 2.71-2.79 (1H, m, CH(CH<sub>3</sub>)<sub>2</sub>), 3.03 (1H, t, *J* = 12.8 Hz, CH<sub>2</sub>), 3.94 (1H, d, *J* = 13.2 Hz, CH<sub>2</sub>), 4.58 (1H, d, *J* = 12.8 Hz, CH<sub>2</sub>), 6.90 (1H, d, *J* = 8.0 Hz, ArH), 7.04 (1H, d, *J* = 8.0 Hz, ArH), 7.05-7.08 (1H, m, ArH), 7.15-7.20 (1H, m, ArH), 7.58 (1H, d, *J* = 8.0 Hz, ArH), 7.69 (1H, s, NH), 8.36 (1H, d, *J* = 8.0 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 19.2, 19.5 (CH<sub>3</sub>), 28.4, 29.0 (CH<sub>2</sub>), 29.9 (CH), 40.1 (CH<sub>2</sub>), 44.1 (CH), 44.5 (CH<sub>2</sub>), 118.9, 121.8, 124.6, 125.2 (ArCH), 125.9 (ArC), 127.3, 127.4

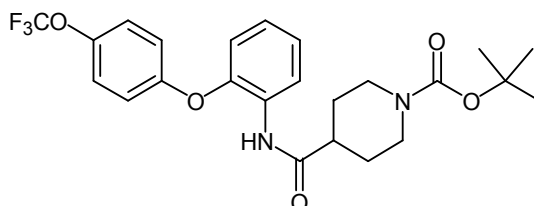
(ArCH), 129.8, 144.5 (ArC), 159.3 (CF<sub>3</sub>), 172.3, 175.3 (CO). HRMS: Calcd for C<sub>23</sub>H<sub>25</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 435.1890, found (M+H)<sup>+</sup> 435.1883.

**1-(3-Methyl-butyryl)-piperidine-4-carboxylic acid [2-(4-trifluoromethyl-phenoxy)-phenyl]-amide, 68**, C<sub>24</sub>H<sub>27</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub>, MW 448.48,



To a solution of piperidine-4-carboxylic acid [2-(4-trifluoromethyl-phenoxy)-phenyl]-amide (0.1 g, 0.27 mmol) in DCM (5 mL) at 0 °C was added TEA (0.2 mL) and isovaleryl carbonyl chloride (0.066 mL, 0.54 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction was quenched with sat. NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL), washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to afford the desired compound as a white waxy solid, 78 mg, 63 % yield. m.p. 63-64 °C, R.f. 0.60 (EtOAc), LCMS: *t*<sub>r</sub> = 4.66 min (50 % MeOH in water at 0.5 mL/min), *m/z* M+H 449.48, HPLC: *t*<sub>r</sub> = 2.35 min (90 % acetonitrile in water at 1.0 mL/min), >99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.94 (6H, d, *J* = 6.0, 2CH<sub>3</sub>), 1.60-1.70 (2H, m, CH<sub>2</sub>), 1.82-1.85 (2H, m, CH<sub>2</sub>), 2.02-2.09 (1H, m, CH), 2.17-2.20 (2H, m, CH<sub>2</sub>), 2.40-2.46 (1H, m, CH), 2.62 (1H, t, *J* = 13.2 Hz, CH<sub>2</sub>), 3.03 (1H, t, *J* = 14.4 Hz, CH<sub>2</sub>), 3.88 (1H, d, *J* = 7.6 Hz, CH<sub>2</sub>), 4.59 (1H, d, *J* = 13.2 Hz, CH<sub>2</sub>), 6.91 (1H, d, *J* = 7.6 Hz, ArH), 7.05 (2H, d, *J* = 8.4 Hz, ArH), 7.08-7.09 (1H, m, ArH), 7.65 (1H, s, NH), 8.38 (1H, dd, *J* = 0.8, 8.0 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 22.63, 22.73 (CH<sub>3</sub>), 25.74 (CH), 28.48, 28.95, 40.78, 42.05 (CH<sub>2</sub>), 44.08 (CH), 45.05 (CH<sub>2</sub>), 117.79, 118.93, 121.75, 124.64, 125.28, 127.35 (ArCH), 127.38, 127.42, 129.81, 144.45 (ArC), 159.32 (CF<sub>3</sub>), 170.93, 172.23 (C=O). HRMS: Calcd for C<sub>24</sub>H<sub>27</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 449.2058, found (M+H)<sup>+</sup> 449.2036. Anal. Calcd for C<sub>24</sub>H<sub>27</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub>: C 64.27, H 6.07, N 6.25 %. Found: C 64.5, H 6.17, N 6.39 %.

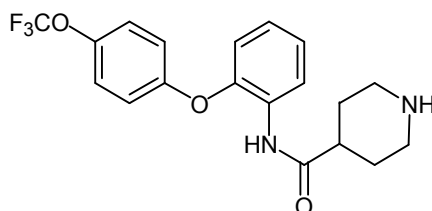
**4-[2-(4-Trifluoromethoxy-phenoxy)-phenylcarbamoyl]-piperidine-1-carboxylic acid tert-butyl ester, 47**, C<sub>24</sub>H<sub>27</sub>F<sub>3</sub>N<sub>2</sub>O<sub>5</sub> MW 480.49,



Using the general procedure for the formation of the amide linker the desired compound was obtained as a yellow wax, 1.59 g, 57 % yield. R.f. 0.65 (DCM), LCMS: *t*<sub>r</sub> = 5.24

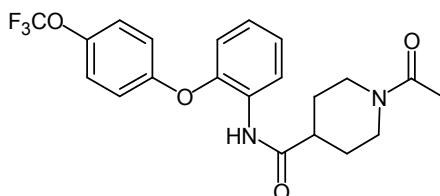
min (50 % to 95 % MeOH: water over 5 min, at 1.0 mL/min),  $m/z$  M+H 479.38, HPLC:  $t_r$  = 3.59 min (90 % acetonitrile in water at 1.0 mL/min), >99 %.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.44 (9H, s,  $\text{CH}_3$ ), 1.67 (2H, td,  $J$  = 13.4, 4.4 Hz,  $\text{CH}_2$ ), 1.80 (2H, dd,  $J$  = 1.8 Hz,  $\text{CH}_2$ ), 2.30-2.41 (1H, m, CH), 2.74 (2H, t,  $J$  = 11.9 Hz,  $\text{CH}_2\text{N}$ ), 4.12 (2H, d,  $J$  = 11.6 Hz,  $\text{CH}_2\text{N}$ ), 6.85 (1H, dd,  $J$  = 6.7, 1.5 Hz, ArH), 7.03 (2H, d, ArH), 6.99-7.06 (1H, m, ArH), 7.13 (1H, td,  $J$  = 1.5, 6.4 Hz, ArH), 7.20 (2H, d,  $J$  = 1.5, 6.4 Hz ArH), 7.64 (1H, s, NH), 8.40 (1H, d,  $J$  = 8.2 Hz, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta$  28.4 ( $\text{CH}_3$ ), 28.5 ( $\text{CH}_2$ ), 43.1 (CH), 117.9, 119.2, 119.4, 121.4, 121.7 122.9, 124.3, 124.6, 129.6, 144.9, 149.3 (ArC), 154.5 ( $\text{OCF}_3$ ), 154.8, 172.6 (CO). HRMS: Calcd for  $\text{C}_{24}\text{H}_{27}\text{F}_3\text{N}_2\text{O}_5$  (M+H) $^+$  481.1945, found (M+H) $^+$  481.1391. Anal. Calcd for  $\text{C}_{24}\text{H}_{27}\text{F}_3\text{N}_2\text{O}_5$ : C 59.99, H 5.66, N 5.83 %. Found: C 60.0, H 5.68, N 5.80 %.

**Piperidine-4-carboxylic acid [2-(4-trifluoromethoxy-phenoxy)-phenyl]-amide, 48e**,  $\text{C}_{19}\text{H}_{19}\text{F}_3\text{N}_2\text{O}_3$ , MW 380.0,



Using the general procedure for the removal of the *N*-Boc protecting group the desired product was obtained as an off white oil, 0.21 g, 87 % yield. R.f: 0.35 (DCM),  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.55-1.68 (2H, m,  $\text{CH}_2$ ), 1.79 (2H, dd,  $J$  = 2.7, 12.9 Hz,  $\text{CH}_2$ ), 2.30-2.36 (1H, m, CH), 2.61 (2H, td,  $J$  = 2.7, 12.3 Hz,  $\text{NCH}_2$ ), 2.94 (1H, s, NH), 3.10 (2H, td,  $J$  = 3.3, 12.6 Hz,  $\text{NCH}_2$ ), 6.79 (1H, dd,  $J$  = 1.2, 8.1, ArH), 6.92-7.00 (3H, m, ArH), 7.08 (1H, td,  $J$  = 1.5, 7.8 Hz, ArH), 7.13 (2H, d,  $J$  = 8.4 Hz, ArH), 7.62 (1H, s, NH), 8.34 (1H, dd,  $J$  = 1.2, 8.1 Hz, ArH).

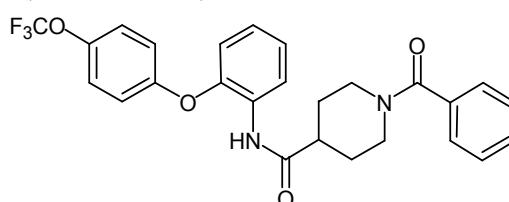
**1-Acetyl-piperidine-4-carboxylic acid [2-(4-trifluoromethoxy-phenoxy)-phenyl]-amide, 69**,  $\text{C}_{21}\text{H}_{21}\text{F}_3\text{N}_2\text{O}_4$ , MW 422.41



To a solution of piperidine-4-carboxylic acid [2-(4-trifluoromethoxy-phenoxy)-phenyl]-amide (105 mg, 0.28 mmol) in DCM (8 mL) at 0 °C was added acetyl chloride (0.04 mL, 0.56 mmol) and TEA (0.18 mL). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction was quenched with sat.  $\text{NaHCO}_3$  (15 mL), extracted with DCM (2 x 20 mL), washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried ( $\text{MgSO}_4$ ), filtered and evaporated *in*

*vacuo*. The crude mixture was purified using flash chromatography (0-5 % methanol in ethyl acetate), to afford the desired compound as a white solid, 55 mg, 47 % yield. m.p. 44-46 °C, R.f. 0.45 (DCM), LCMS:  $t_r$  = 1.08 min (95 % MeOH in water at 1.0 mL/min),  $m/z$  M+H 423.48, HPLC:  $t_r$  = 2.12 min (90 % acetonitrile in water at 1.0 mL/min), 97 %, HRMS: Calcd for  $C_{21}H_{21}F_3N_2O_4$  (M+H)<sup>+</sup> 423.1526, found (M+H)<sup>+</sup> 423.1525, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.54-1.72 (2H, m, CH<sub>2</sub>), 1.78-1.86 (2H, m, CH<sub>2</sub>), 2.02 (3H, s, CH<sub>3</sub>), 2.35-2.45 (1H, m, CH), 2.60 (1H, td,  $J$  = 3.0, 14.7 Hz, CH<sub>2</sub>), 2.98-3.08 (1H, m, CH<sub>2</sub>), 3.79 (1H, d,  $J$  = 13.5 Hz, CH<sub>2</sub>), 4.52 (1H, d,  $J$  = 13.2 Hz, CH<sub>2</sub>), 6.79 (1H, dd,  $J$  = 1.2, 8.1 Hz, ArH), 6.92-7.01 (3H, m, ArH), 7.08 (1H, td,  $J$  = 1.2, 7.8 Hz, ArH), 7.15 (2H, dd,  $J$  = 0.6, 9.0 Hz, ArH), 7.26 (1H, s, NH), 8.32 (1H, dd,  $J$  = 1.2, 8.1 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 21.4 (CH<sub>2</sub>), 28.37, 28.8, 40.7 (CH<sub>2</sub>), 44.1 (CH), 45.6 (CH<sub>2</sub>), 117.9, 119.4, 121.4, 122.9, 124.5, 124.6 (ArCH), 129.4, 145.0, 154.8 (ArC), 168.9, 172.1 (CO). <sup>19</sup>F NMR (CDCl<sub>3</sub>, 374 MHz): δ -58.26 (s, OCF<sub>3</sub>). Anal. Calcd for  $C_{21}H_{21}F_3N_2O_4$ : C 59.71, H 5.01, N 6.63 %. Found: C 58.0, H 4.93, N 6.37 %.

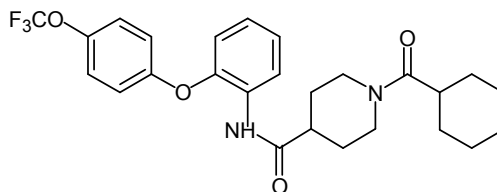
**1-Benzoyl-piperidine-4-carboxylic acid [2-(4-trifluoromethoxy-phenoxy)-phenyl]-amide, 70**,  $C_{26}H_{23}F_3N_2O_4$ , MW 484.45,



To a solution of piperidine-4-carboxylic acid [2-(4-trifluoromethoxy-phenoxy)-phenyl]-amide (105 mg, 0.28 mmol) in DCM (8 mL) at 0 °C, was added benzoyl chloride (0.064 mL, 0.56 mmol) and TEA (0.18 mL). The reaction mixture was allowed to warm to room temperature, and stirred for 30 min. The reaction was quenched with sat. NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL), washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-5 % methanol in DCM), to afford the title compound a white wax, 105 mg, 78 % yield. R.f. 0.45 (DCM), LCMS:  $t_r$  = 4.9 min (50% MeOH in water at 0.5 mL/min),  $m/z$  M<sup>+</sup>H 485.43, HPLC:  $t_r$  = 5.3 (90 % acetonitrile in water at 1.0 mL/min), 98 %, HRMS: Calcd for  $C_{26}H_{23}F_3N_2O_4$  (M+H)<sup>+</sup> 485.1683, found (M+H)<sup>+</sup> 485.1685, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.74 (4H, s, 2CH<sub>2</sub>), 2.39-2.49 (1H, m, CH), 2.90 (2H, s, CH<sub>2</sub>), 3.77 (1H, s, CH<sub>2</sub>), 4.63 (1H, s, CH<sub>2</sub>), 6.80 (1H, dd,  $J$  = 1.2, 8.1 Hz, ArH), 6.92-6.98 (1H, m, ArH), 6.95 (2H, m, ArH), 7.09 (1H, td,  $J$  = 1.5, 7.8 Hz, ArH), 7.15 (2H, dd,  $J$  = 0.6, 9.0 Hz, ArH), 7.30-7.36 (5H, m, ArH), 7.63 (1H, s, NH), 8.32 (1H, d,  $J$  = 7.2 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 28.6, 28.9, 41.4 (CH<sub>2</sub>), 44.1 (CH), 46.9 (CH<sub>2</sub>), 117.9, 119.4, 121.5, 122.9, 124.5, 124.6, 128.5 (ArCH), 129.4 (ArC), 129.7 (ArCH), 135.8, 145.0, 145.4, 154.7 (ArC), 170.5, 172.1 (CO). <sup>19</sup>F NMR (CDCl<sub>3</sub>, 374 MHz): δ -58.25

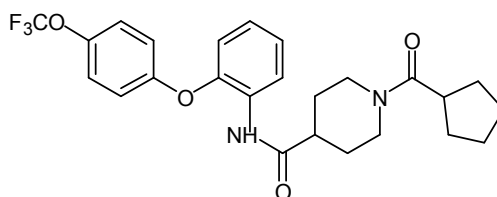
(s, OCF<sub>3</sub>). Anal. Calcd for C<sub>26</sub>H<sub>23</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub>: C 64.46, H 4.79, N 5.78 %. Found: C 63.7, H 4.76, N 5.57 %.

**1-Cyclohexanecarbonyl-piperidine-4-carboxylic acid [2-(4-trifluoromethoxy-phenoxy)-phenyl]-amide, 71**, C<sub>26</sub>H<sub>29</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub>, MW 490.51



To a solution of piperidine-4-carboxylic acid [2-(4-trifluoromethoxy-phenoxy)-phenyl]-amide (0.1 g, 0.3 mmol) in DCM (5 mL) at 0 °C was added TEA (0.2 mL) and cyclohexane carbonyl chloride (0.072 mL, 0.52 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction was quenched with sat. NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL), washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to afford the title compounds as a white waxy solid, 98 mg, 76 % yield. m.p. 98-100 °C, R.f 0.70 (EtOAc), LCMS: *t<sub>r</sub>* = 5.08 min (50 % MeOH in water at 0.5 mL/min), *m/z* M+H 489.33, HPLC: *t<sub>r</sub>* = 2.57 min (90 % acetonitrile in water at 1.0 mL/min), 98 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.47-1.86 (12H, m, 6CH<sub>2</sub>), 2.38-2.52 (2H, m, 2CH), 2.61 (1H, t, *J* = 11.6 Hz, CH<sub>2</sub>), 3.03 (1H, t, *J* = 12.2 Hz, CH<sub>2</sub>), 3.94 (1H, d, *J* = 13.1 Hz, CH<sub>2</sub>), 4.62 (1H, d, *J* = 12.9 Hz, CH<sub>2</sub>), 6.85 (1H, dd, *J* = 1.5, 8.2 Hz, ArH), 7.00 (2H, d, *J* = 9.2 Hz, ArH), 6.99-7.03 (1H, m, ArH), 7.14 (1H, td, *J* = 1.5, 7.9 Hz, ArH), 7.20 (2H, d, *J* = 8.4 Hz, ArH), 7.68 (1H, s, NH), 8.39 (1H, d, *J* = 8.2 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 25.81, 25.84, 28.57, 29.25, 29.52 (CH<sub>2</sub>), 40.46 (CH), 40.94 (CH<sub>2</sub>), 44.38 (CH), 44.63 (CH<sub>2</sub>), 117.92, 119.34, 121.38, 122.92, 124.43, 124.67, 129.47, 129.47, 144.98, 145.28 (ArC), 154.79 (OCF<sub>3</sub>), 172.22, 174.53 (C=O). HRMS: Calcd for C<sub>26</sub>H<sub>29</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub> (M+H)<sup>+</sup> 491.2152, found (M+H)<sup>+</sup> 491.2154. Anal. Calcd for C<sub>26</sub>H<sub>29</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub>: C 63.66, H 5.96, N 5.71. Found C 63.6, H 5.86, N 5.58.

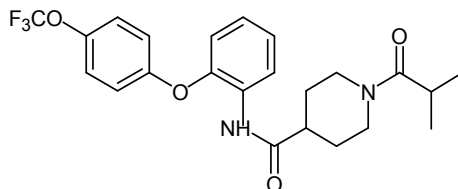
**1-Cyclopentanecarbonyl-piperidine-4-carboxylic acid [2-(4-trifluoromethoxy-phenoxy)-phenyl]-amide, 72**, C<sub>25</sub>H<sub>27</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub>, MW 476.49,



To a solution of piperidine-4-carboxylic acid [2-(4-trifluoromethoxy-phenoxy)-phenyl]-amide (0.1 g, 0.3 mmol) in DCM (5 mL) at 0 °C was added TEA (0.2 mL) and

cyclopentane carbonyl chloride (0.066 mL, 0.52 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction was quenched with sat. NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL), washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to afford the title compound as a white waxy solid, 85 mg, 68 % yield. m.p. 48-50 °C, R.f. 0.68 (EtOAc), LCMS: *t<sub>r</sub>* = 5.0 min (50 % MeOH in water at 0.5 mL/min), *m/z* M+H 477.56, HPLC: *t<sub>r</sub>* = 2.5 min (90 % acetonitrile in water at 1.0 mL/min), 99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.53-1.91 (12H, m, 6CH<sub>2</sub>), 2.41-2.52 (1H, m, CH), 2.64 (1H, td, *J* = 2.7, 13.4 Hz, CH<sub>2</sub>), 2.80-2.91 (1H, m, CH), 3.05 (1H, td, *J* = 2.5, 13.6 Hz, CH<sub>2</sub>), 4.01 (1H, d, *J* = 13.4 Hz, CH<sub>2</sub>), 4.62 (1H, d, *J* = 13.4 Hz, CH<sub>2</sub>), 6.85 (1H, dd, *J* = 1.2, 7.9 Hz, ArH), 7.00 (2H, d, *J* = 9.2 Hz, ArH), 7.00-7.06 (1H, m, ArH), 7.14 (1H, td, *J* = 1.8, 7.9 Hz, ArH), 7.20 (2H, d, *J* = 0.8, 9.1 Hz, ArH), 7.69 (1H, s, NH), 8.39 (1H, dd, *J* = 1.5, 8.2 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 25.99, 28.56, 29.09, 30.22 (CH<sub>2</sub>), 41.07 (CH), 41.16 (CH<sub>2</sub>), 44.39 (CH), 44.76 (CH<sub>2</sub>), 117.91, 119.41, 121.36, 122.91, 124.41, 124.42, 124.67, 129.49, 145.00, 145.27 (ArC), 154.78 (OCF<sub>3</sub>), 172.24, 174.44 (C=O). HRMS: Calcd for C<sub>25</sub>H<sub>27</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub> (M+H)<sup>+</sup> 477.1996, found (M+H)<sup>+</sup> 477.1987. Anal. Calcd for C<sub>25</sub>H<sub>27</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub>: C 63.02, H 5.71, N 5.88. Found C 63.2, H 5.77, N 5.86.

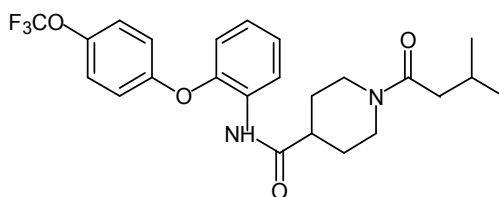
**1-Isobutyryl-piperidine-4-carboxylic acid [2-(4-trifluoromethoxy-phenoxy)-phenyl]-amide, 73**, C<sub>23</sub>H<sub>25</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub>, MW 450.45,



To a solution of piperidine-4-carboxylic acid [2-(4-trifluoromethoxy-phenoxy)-phenyl]-amide (0.1 g, 0.26 mmol) in DCM (5 mL) at 0 °C was added TEA (0.2 mL) and isobutyryl chloride (0.057 mL, 0.52 mmol). The reaction mixture was allowed to warm to room temperature, and stirred for 30 min. The reaction was quenched with sat. NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL), washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in-vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to afford the title compound as a white waxy solid, 89 mg, 75 % yield. m.p. 101-103 °C, R.f. 0.56 (EtOAc), LCMS: *t<sub>r</sub>* = 5.37 min (50 % MeOH in water at 0.5 mL/min), *m/z* M+H 449.35, HPLC: *t<sub>r</sub>* = 2.86 min (90 % acetonitrile in water at 1.0 mL/min), 98 %, HRMS: Calcd for C<sub>23</sub>H<sub>25</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub> (M+H)<sup>+</sup> 451.1839, found (M+H)<sup>+</sup> 451.1839, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.08-1.12 (6H, m, CH<sub>3</sub>), 1.62-1.78 (2H, m, CH<sub>2</sub>), 1.86-1.89 (2H, m, CH<sub>2</sub>), 2.41-2.52 (1H, m, CH<sub>2</sub>), 2.59-

2.72 (1H, m, CH<sub>2</sub>), 2.75-2.85 (1H, m, CH<sub>2</sub>), 3.06 (1H, t, *J* = 12.4 Hz, CH<sub>2</sub>), 3.98 (1H, d, *J* = 12.8 Hz, CH<sub>2</sub>), 4.63 (1H, d, *J* = 13.1 Hz, CH<sub>2</sub>), 6.84 (1H, dd, *J* = 1.5, 7.9 Hz, ArH), 7.01 (2H, d, *J* = 9.1 Hz, ArH), 6.98-7.07 (1H, m, ArH), 7.14 (1H, td, *J* = 1.5, 7.7 Hz, ArH), 7.20 (2H, d, *J* = 8.9 Hz, ArH), 7.67 (1H, s, NH), 8.39 (1H, dd, *J* = 1.5, 8.2 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 19.28, 19.53 (CH<sub>3</sub>), 28.57, 29.15 (CH<sub>2</sub>), 30.07 (CH), 41.04 (CH<sub>2</sub>), 44.35 (CH), 44.66 (CH<sub>2</sub>), 117.91, 119.41, 121.36, 122.91, 124.43, 124.67, 129.47, 144.99, 149.28 (ArC), 154.78 (CF<sub>3</sub>), 172.20, 175.34 (C=O). Anal. Calcd for C<sub>23</sub>H<sub>25</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub>: C 61.33, H 5.59, N 6.22. Found C 61.3, H 5.61, N 5.93.

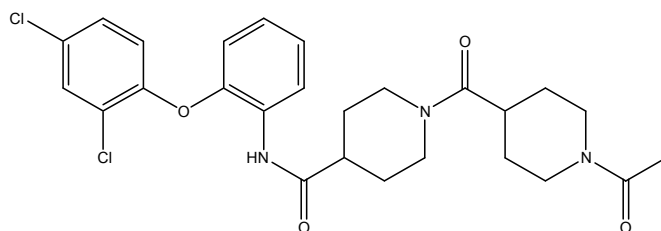
**1-(3-Methyl-butyl)-piperidine-4-carboxylic acid [2-(4-trifluoromethoxy-phenoxy)-phenyl]-amide, 74**, C<sub>24</sub>H<sub>27</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub>, MW 464.48,



To a solution of piperidine-4-carboxylic acid [2-(4-trifluoromethoxy-phenoxy)-phenyl]-amide (0.1 g, 0.3 mmol) in DCM (5 mL) at 0 °C was added TEA (0.2 mL) and isovaleryl chloride (0.066 mL, 0.52 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction was quenched with sat. NaHCO<sub>3</sub> (15 mL), extracted with DCM (2 x 20 mL), washed with hydrochloric acid (1M, 20 mL) and brine (20 mL). The organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in-vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to afford the title compound as a white waxy solid, 82 mg, 67 % yield. m.p. 82-85 °C, R.f. 0.60 (EtOAc), LCMS: *t*<sub>r</sub> = 5.38 min (50 % MeOH in water at 0.5 mL/min), *m/z* M+H 463.33, HPLC: *t*<sub>r</sub> = 2.42 min (90 % acetonitrile in water at 1.0 mL/min), 98 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 0.95 (6H, d, *J* = 6.4 Hz, CH<sub>3</sub>), 1.63-1.76 (2H, m, CH<sub>2</sub>), 1.85-1.90 (2H, m, CH<sub>2</sub>), 2.01-2.16 (1H, m, CH), 2.19 (2H, m, CH<sub>2</sub>), 2.40-2.51 (1H, m, CH), 2.64 (1H, td, *J* = 3.0, 13.4 Hz, CH<sub>2</sub>), 2.99-3.10 (1H, m, CH<sub>2</sub>), 3.91 (1H, d, *J* = 14.1 Hz, CH<sub>2</sub>), 4.63 (1H, d, *J* = 13.6 Hz, CH<sub>2</sub>), 6.85 (1H, dd, *J* = 1.5, 8.2 Hz, ArH), 7.00 (2H, d, *J* = 9.2 Hz, ArH), 6.97-7.07 (1H, m, ArH), 7.14 (1H, td, *J* = 1.5, 7.7 Hz, ArH), 7.20 (2H, d, *J* = 9.2 Hz, ArH), 7.67 (1H, s, NH), 8.39 (1H, dd, *J* = 1.5, 8.2 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 22.67, 22.77 (CH<sub>3</sub>), 25.77 (CH), 28.57, 29.02, 40.84, 42.01 (CH<sub>2</sub>), 44.25 (CH), 45.11 (CH<sub>2</sub>), 117.91, 119.41, 121.38, 122.92, 124.44, 124.67, 129.47, 145.01 (ArC), 145.30 (OCF<sub>3</sub>), 154.78, 170.95 (C=O). HRMS: Calcd for C<sub>24</sub>H<sub>27</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub> (M+H)<sup>+</sup> 465.1996, found (M+H)<sup>+</sup> 465.1975. Anal. Calcd for C<sub>24</sub>H<sub>27</sub>F<sub>3</sub>N<sub>2</sub>O<sub>4</sub>: C 62.06, H 5.86, N 6.03. Found C 62.1, H 5.96, N 6.01.

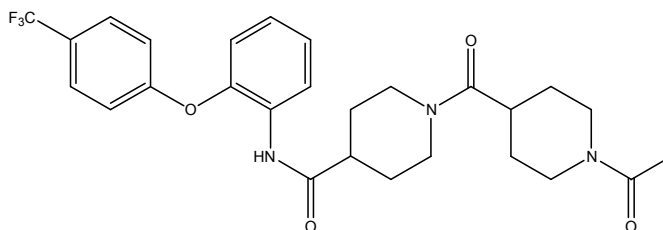


**1-(1-Acetyl-piperidine-4-carbonyl)-piperidine-4-carboxylic acid [2-(2,4-dichloro-phenoxy)-phenyl]-amide, 76**, C<sub>26</sub>H<sub>29</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>4</sub>, MW 518.43,



Piperidine-4-carboxylic acid [2-(2,4-dichloro-phenoxy)-phenyl]-amide (**43a**, 100 mg, 0.27 mmol) was dissolved in anhydrous DCM (10 mL) and stirred under nitrogen. To this was added 1-acetylpiperidine-4-carbonyl chloride (0.1 g, 0.54 mmol) and TEA (0.17 mL) this was stirred at room temperature for 1 h. NaHCO<sub>3</sub> (20 mL) was then added, and extracted with DCM (2 x 20 mL) and washed with HCl (1M, 20 mL). The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-5 % MeOH in DCM) to afford the title compound as a white solid, 0.121 g, 86 % yield. R.f. 0.59 (5 % MeOH in DCM), m.p. 99-101 °C, LCMS: *t<sub>r</sub>* = 1.16 min (95 % MeOH in water), *m/z* M-H 516.43, HPLC: *t<sub>r</sub>* = 1.77 min (90 % acetonitrile in water), 99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.69-1.74 (8H, m, 4CH<sub>2</sub>), 2.07 (3H, s, CH<sub>3</sub>), 2.66-2.75 (4H, m, 2CH<sub>2</sub>), 3.04-3.17 (2H, m, CH<sub>2</sub>), 3.83-3.99 (2H, m, CH<sub>2</sub>), 4.55 (2H, d, *J* = 12.4Hz, CH<sub>2</sub>), 6.71 (1H, dd, *J* = 1.5, 8.2Hz, ArH), 6.94 (1H, d, *J* = 8.7Hz, ArH), 7.00 (1H, dd, *J* = 1.5, 7.9Hz, ArH), 7.12 (1H, td, *J* = 1.2, 7.7Hz, ArH), 7.21 (1H, dd, *J* = 2.5, 8.7Hz, ArH), 7.48 (1H, d, *J* = 2.5, ArH), 7.80 (1H, s, NH), 8.37 (1H, dd, *J* = 1.5, 8.2Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): δ 21.6 (CH<sub>3</sub>), 28.3, 28.7, 28.9(CH<sub>2</sub>), 29.2 (CH), 41.0, 41.3 (CH<sub>2</sub>), 44.2 (CH), 44.8, 45.8 (CH<sub>2</sub>), 116.6, 121.5, 124.4, 124.6, 128.5 (ArCH), 128.9, 130.3 (ArC), 130.7 (ArCH), 145.3 (ArC), 172.5 (CO). Anal. Calcd for C<sub>26</sub>H<sub>29</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>4</sub>.H<sub>2</sub>O: C 58.2, H 5.82, N 7.8 %. Found: C 58.8, H 5.6, N 7.8 %. HRMS: Calcd for C<sub>26</sub>H<sub>29</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>4</sub> (M+H)<sup>+</sup> 518.1608, found (M+H)<sup>+</sup> 518.1617.

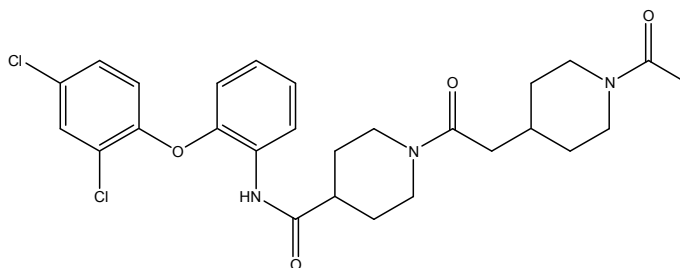
**1-(1-Acetyl-piperidine-4-carbonyl)-piperidine-4-carboxylic acid [2-(4-trifluoromethyl-phenoxy)-phenyl]-amide, 77**, C<sub>27</sub>H<sub>30</sub>F<sub>3</sub>N<sub>3</sub>O<sub>4</sub>, MW 517.54,



Piperidine-4-carboxylic acid [2-(4-trifluoromethyl-phenoxy)-phenyl]-amide (**43d**, 100 mg, 0.27 mmol) was dissolved in anhydrous DCM (10 mL) and stirred under nitrogen. To this was added 1-acetylpiperidine-4-carbonyl chloride (100 mg, 0.54 mmol) and TEA (0.17 mL) this was then stirred at room temperature for 40 min. NaHCO<sub>3</sub> (20 mL)

was then added and extracted with DCM (2 x 20 mL) and washed with HCl (1M, 20 mL). The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-10 % MeOH in DCM ) to afford the title compound as a white solid, 119 mg, 84 % yield. R.f. 0.58 (5 % MeOH in DCM), m.p. 84-89 °C, LCMS:  $t_r$  = 1.11 min (95 % MeOH in water),  $m/z$  M-H 516.64, HPLC:  $t_r$  = 1.30 min (90 % acetonitrile in water), >99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  1.59-1.85 (8H, m, 4CH<sub>2</sub>), 2.06 (3H, s, CH<sub>3</sub>), 2.46 (1H, t,  $J$  = 8.0 Hz, CH), 2.62-2.72 (3H, m, CH and CH<sub>2</sub>), 3.08 (2H, t,  $J$  = 12.4 Hz, CH<sub>2</sub>), 3.83-3.93 (2H, m, CH<sub>2</sub>), 4.55 (2H, d,  $J$  = 12.4 Hz, CH<sub>2</sub>), 6.91 (1H, dd,  $J$  = 0.8, 8.0 Hz, ArH), 7.04-7.09 (3H, m, ArH), 7.16-7.20 (1H, m, ArH), 7.59 (2H, d,  $J$  = 8.8 Hz, ArH), 7.68 (1H, s, NH), 8.35 (1H, d,  $J$  = 8.0 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta$  21.4 (CH<sub>3</sub>), 28.4, 28.7, 29.0, 29.1 (CH<sub>2</sub>), 38.1 (CH), 40.84 (CH<sub>2</sub>), 43.9 (CH), 44.6, 45.7 (CH<sub>2</sub>), 117.8, 119.0, 121.9, 124.7, 125.3, 127.4 (ArCH), 129.8, 144.5, 159.5, 168.8 (ArC), 172.0, 172.1, 172.3 (CO). HRMS: Calcd for C<sub>27</sub>H<sub>30</sub>F<sub>3</sub>N<sub>3</sub>O<sub>4</sub> (M+H)<sup>+</sup> 518.2261, found (M+H)<sup>+</sup> 518.2262.

**1-[2-(1-Acetyl-piperidin-4-yl)-acetyl]-piperidine-4-carboxylic acid [2-(2,4-dichlorophenoxy)-phenyl]-amide, 78**, C<sub>27</sub>H<sub>31</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>4</sub>, MW 532.46,



(1-Acetyl-piperidin-4-yl)-acetic acid (50 mg, 0.27 mmol) was dissolved in anhydrous DCM (20 mL) and stirred under nitrogen. To this was added *N*-4-dimethylaminopyridine (cat.), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (160 mg, 0.9 mmol) and TEA (0.05 mL). The reaction was stirred at room temperature for 30 min. To this was added piperidine-4-carboxylic acid [2-(2,4-dichlorophenoxy)-phenyl]-amide (**50a**, 100 mg, 0.27 mmol) and the reaction stirred for 2 days. The reaction mixture was diluted with DCM (30 mL), washed with HCl (1M, 20 mL), NaHCO<sub>3</sub> (sat. 20 mL) and brine (20 mL). The organic layers were combined and dried (MgSO<sub>4</sub>) and evaporated *in vacuo* to afford a white wax, 24 mg, 17 % yield. R.f. 0.45 (10% MeOH in DCM), LCMS:  $t_r$  = 1.18 min (95 % MeOH in water),  $m/z$  M-H 530.42, 532.51, HPLC:  $t_r$  = 1.60 min (90 % acetonitrile in water), 99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  1.07-1.18 (2H, m, CH<sub>2</sub>), 1.68-1.75 (4H, m, CH<sub>2</sub>), 1.85 (1H, d,  $J$  = 12.8Hz, CH<sub>2</sub>), 1.91-1.92 (2H, m, CH<sub>2</sub>), 2.06 (3H, s, CH<sub>3</sub>), 2.08-2.31 (2H, m,  $\frac{1}{2}$ CH<sub>2</sub> and CH), 2.49-2.59 (2H, m,  $\frac{1}{2}$ CH<sub>2</sub> and CH), 2.70 (1H, t,  $J$  = 12.8Hz,  $\frac{1}{2}$ CH<sub>2</sub>), 3.02-3.12 (2H, m, CH<sub>2</sub>), 3.77 (1H, d,  $J$  = 14Hz, CH<sub>2</sub>), 3.91 (1H, d,  $J$  = 13.2Hz,  $\frac{1}{2}$ CH<sub>2</sub>), 4.59 (2H, dd,  $J$  =

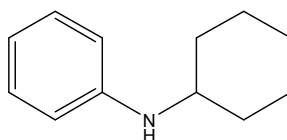
4.4, 11.2Hz, CH<sub>2</sub>), 6.72 (1H, dd,  $J = 1.2, 8.4$ Hz, ArH), 6.94 (1H, d,  $J = 8.8$ Hz, ArH), 7.00 (1H, td,  $J = 1.6, 8.0$ Hz, ArH), 7.13 (1H, td,  $J = 1.2, 7.6$ Hz, ArH), 7.22 (1H, dd,  $J = 2.8, 8.8$ Hz, ArH), 7.49 (1H, d,  $J = 2.4$ Hz, ArH), 7.78 (1H, s, NH), 8.37 (1H, d,  $J = 7.6$ Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz):  $\delta$  21.5 (CH<sub>3</sub>), 28.5, 28.6, 28.9, 32.0, 32.7, 32.8 (CH<sub>2</sub>), 33.1 (CH), 39.3, 39.4, 41.0, 41.7 (CH<sub>2</sub>), 44.1(CH), 44.9, 46.5 (CH<sub>2</sub>), 116.5, 121.4, 121.5, 124.3, 124.5 (ArCH), 126.3 (ArC), 128.4 (ArCH), 128.8, 130.2 (ArC), 130.6 (ArCH), 145.2, 150.3 (ArC), 168.7, 169.8, 172.1 (CO). HRMS: Calcd for C<sub>27</sub>H<sub>31</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>4</sub> (M+H)<sup>+</sup> 532.1764, found (M+H)<sup>+</sup> 532.1764.

## Experimental Details: Chapter 7: Synthesis of the Amine Linked Series of Inhibitors

### General Microwave Experimental Procedure for Ketones

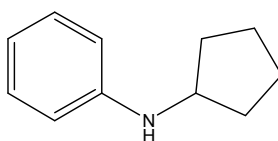
This method is as reported by Bailey *et al.*<sup>85</sup> To a solution of aniline (1 mmol) and ketone (2 mmol) in DCE (2 mL) in a microwave vial, was added NaBH(OAc)<sub>3</sub> (2.5 mmol) and AcOH (3 mmol). The vial was capped and the resulting solution was heated in a CEM Discover<sup>®</sup> microwave for 10 minutes (fixed hold time) at 140 °C. The reaction was quenched with a saturated aqueous solution of NaHCO<sub>3</sub> (10 mL) was then extracted with DCM (3 x 15 mL). The combined organics were dried (MgSO<sub>4</sub>), filtered and concentrated *in vacuo*. Purification by flash chromatography (DCM) then proceeded to afford the desired product.

**Cyclohexylphenylamine, 79**, C<sub>12</sub>H<sub>17</sub>N, MW 175.27,



Using the general microwave experimental procedure for ketones the desired product was isolated, 94 %, LCMS: r.t. 1.31 min (95% MeOH in water), M<sup>+</sup>H: 175.96, HPLC: r.t. 3.04 min (90% acetonitrile in water), 92% pure, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  1.10-1.48 (5H, m, CH<sub>2</sub>), 1.65-1.90 (3H, m, CH<sub>2</sub>), 2.05-2.15 (2H, m, CH<sub>2</sub>), 3.20-3.35 (1H, m, CH), 3.53 (1H, s, NH), 6.59-6.75 (3H, m, ArH), 7.15-7.22 (2H, m, ArH). This compound has been previously synthesised via a different route, by Reddy *et al.*<sup>140</sup>

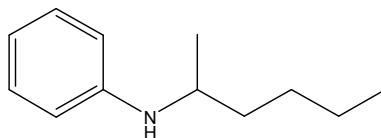
**Cyclopentylphenylamine, 80**, C<sub>11</sub>H<sub>15</sub>N, MW 161.24,



Using the general microwave experimental procedure for ketones the desired product was isolated, 88 %, LCMS: r.t. 1.45 min (95% MeOH in water), M<sup>+</sup>H: 162.16, HPLC:

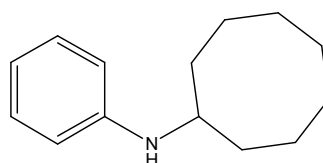
r.t. 6.02 min (90% acetonitrile in water), 99% pure,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.54-1.79 (6H, m,  $\text{CH}_2$ ), 2.04-2.15 (2H, m,  $\text{CH}_2$ ), 3.69 (1H, s, NH), 3.82-3.86 (1H, m, CH), 6.64-6.82 (3H, m, ArH), 7.20-7.28 (2H, m, ArH). This compound has been previously synthesised via a different route, by Reddy *et al.*<sup>140</sup>

**(1-Methylpentyl)phenylamine, 81**,  $\text{C}_{12}\text{H}_{19}\text{N}$ , MW 177.29,



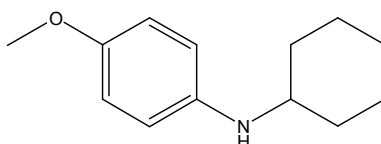
Using the general microwave experimental procedure for ketones the desired product was isolated, 92 %, LCMS: r.t. 1.34 min (95% MeOH in water),  $\text{M}^+\text{H}$ : 178.05, HPLC: r.t. 3.19 min (90% acetonitrile in water), 97% pure,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  0.92-1.01 (3H, m,  $\text{CH}_3\text{CH}_2$ ), 1.20-1.25 (3H, m,  $\text{CH}_3\text{CH}$ ), 1.35-1.70 (6H, m,  $\text{CH}_2$ ), 3.48-3.51 (2H, m, CH and NH), 6.62-6.73 (3H, m, ArH), 7.21-7.30 (2H, m, ArH). This compound has been previously synthesised via a different route, by Barluenga *et al.*<sup>141</sup>

**Cyclooctylphenylamine, 82**,  $\text{C}_{14}\text{H}_{21}\text{N}$ , MW203.32,



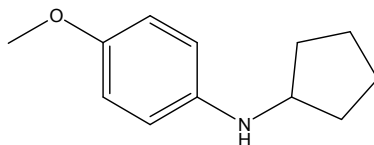
Using the general microwave experimental procedure for ketones the desired product was isolated, 68 %, LCMS: r.t. 1.62 min (95% MeOH in water),  $\text{M}^+\text{H}$ : 204.03, HPLC: r.t. 9.16 min (90% acetonitrile in water), 93% pure,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.55-2.04 (14H, m,  $\text{CH}_2$ ), 3.56-3.58 (2H, m, CH and NH), 6.55-6.75 (3H, m, ArH), 7.15-7.20 (2H, m, ArH). This compound has been previously synthesised via a different route, by Anderson *et al.*<sup>142</sup>

**Cyclohexyl-(4-methoxyphenyl)amine, 83**,  $\text{C}_{13}\text{H}_{19}\text{NO}$ , MW 205.30,



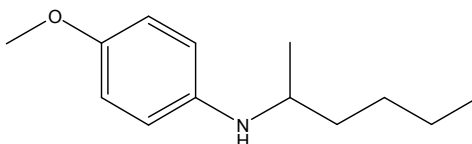
Using the general microwave experimental procedure for ketones the desired product was isolated as an off white solid, 77 %, m.p. 42-45 °C (lit. 41-42 °C),<sup>143</sup> LCMS: r.t. 1.20 min (95% MeOH in water),  $\text{M}^+\text{H}$ : 205.99, HPLC: r.t. 2.72 min (90% acetonitrile in water), 81% pure,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.08-1.38 (5H, m,  $\text{CH}_2$ ), 1.60-1.78 (3H, m,  $\text{CH}_2$ ), 2.02-2.10 (2H, m,  $\text{CH}_2$ ), 3.12-3.20 (2H, m, CH and NH), 3.74 (3H, s,  $\text{OCH}_3$ ), 6.55-6.59 (2H, m, ArH), 6.75-6.78 (2H, m, ArH).

**Cyclopentyl-(4-methoxyphenyl)amine, 84**, C<sub>12</sub>H<sub>17</sub>NO, MW 191.27,



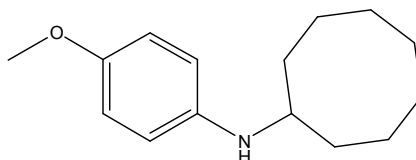
Using the general microwave experimental procedure for ketones the desired product was isolated, 64 %, LCMS: r.t. 1.35 min (95% MeOH in water), M<sup>+</sup>H 192.35, HPLC: r.t. 5.41 min (90% acetonitrile in water), 99% pure, HRMS: Calculated: 192.1383, Measured: 192.1384, IR (CDCl<sub>3</sub>): 3417 (NH), 2959, 2871, 2835, 2247 (CH), 1510, 1235 (Ar), 1039 cm<sup>-1</sup> (CO). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.40-1.75 (6H, m, CH<sub>2</sub>), 1.95-2.01 (2H, m, CH<sub>2</sub>), 3.35 (1H, s, NH), 3.71-3.74 (4H, m, OCH<sub>3</sub> and CH), 6.57-6.60 (2H, m, ArH), 6.77-6.80 (2H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): δ 24.2, 33.7 (CH<sub>2</sub>), 55.6 (OCH<sub>3</sub>), 55.9 (CH), 114.7, 114.9 (ArCH), 142.4, 151.9 (ArC). This compound was previously synthesised via a different route, by Xu *et al.*<sup>144</sup>

**(4-Methoxyphenyl)-(1-methylpentyl)amine, 85**, C<sub>13</sub>H<sub>21</sub>NO, MW 207.31,



Using the general microwave experimental procedure for ketones the desired product was isolated, 87 %, LCMS: r.t. 1.25 min (95% MeOH in water), M<sup>+</sup>H: 207.88, HPLC: r.t. 3.22 min (90% acetonitrile in water), 94% pure, HRMS: Calculated: 208.1696, Measured: 208.1694, IR (CDCl<sub>3</sub>): 2960, 2933, 2873, 2248 (CH), 1510, 1465 (Ar), 1234, 1040 cm<sup>-1</sup> (CO). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 0.93 (3H, t, *J* = 7.7 Hz, CH<sub>3</sub>CH<sub>2</sub>), 1.16 (3H, d, *J* = 6.4 Hz, CH<sub>3</sub>CH), 1.34-1.39 (6H, m, CH<sub>2</sub>), 3.12 (1H, s, NH), 3.36-3.41 (1H, m, CH), 3.76 (3H, s, OCH<sub>3</sub>), 6.56-6.58 (2H, m, ArH), 6.78-6.81 (2H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): δ 14.3 (CH<sub>3</sub>CH<sub>2</sub>), 20.9 (CH<sub>3</sub>CH), 22.9, 28.5, 37.0 (CH<sub>2</sub>), 49.6 (CH), 55.9 (OCH<sub>3</sub>), 114.8, 115.0 (ArCH), 142.1, 151.9 (ArC).

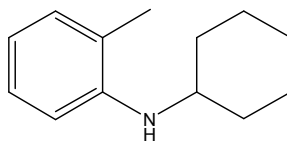
**Cyclooctyl-(4-methoxyphenyl)amine, 86**, C<sub>15</sub>H<sub>23</sub>NO, MW 233.35,



Using the general microwave experimental procedure for ketones the desired product was isolated, 64 %, LCMS: r.t. 1.70 min (95% MeOH in water), M<sup>+</sup>H: 234.44, HPLC: r.t. 7.91 min (90% acetonitrile in water), 95% pure, IR (CDCl<sub>3</sub>): 2927, 2856, 2254 (CH), 1511, 1466 (Ar), 1130, 1039 cm<sup>-1</sup> (CO), <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.41-1.90 (14H, m, CH<sub>2</sub>), 3.21 (1H, s, NH), 3.38-3.45 (1H, m, CH), 3.72 (3H, s, OCH<sub>3</sub>), 6.51-6.55 (2H, m, ArH), 6.75-6.78 (2H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): δ 24.2, 26.0, 27.2,

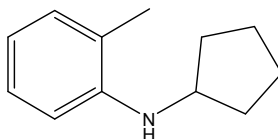
32.7 (CH<sub>2</sub>), 53.7 (OCH<sub>3</sub>), 55.9 (CH), 115.0, 115.1 (ArCH), 141.5, 151.9 (ArC). HRMS: Calcd for C<sub>15</sub>H<sub>23</sub>NO (M+H)<sup>+</sup> 234.1852, found (M+H)<sup>+</sup> 234.1852.

**Cyclohexyl-*o*-tolylamine, 87**, C<sub>13</sub>H<sub>19</sub>N, MW 189.30,



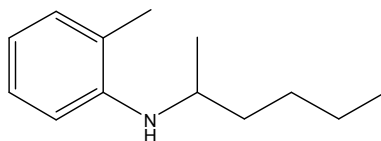
Using the general microwave experimental procedure for ketones the desired product was isolated as a colourless oil, 95 %, LCMS: r.t. 1.66 min (95% MeOH in water), M<sup>+</sup>H 189.86, HPLC: r.t. 4.10 min (90% acetonitrile in water), 90% pure, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.20-1.55 (5H, m, CH<sub>2</sub>), 1.70-1.90 (3H, m, CH<sub>2</sub>), 2.13-2.19 (5H, m, CH<sub>2</sub> and CH<sub>3</sub>), 3.39-3.42 (2H, m, NH and CH), 6.67-6.72 (2H, m, ArH), 7.10-7.17 (2H, m, ArH). This compound has been previously synthesised via a different route, by Mićović *et al.*<sup>70</sup>

**Cyclopentyl-*o*-tolylamine, 88**, C<sub>12</sub>H<sub>17</sub>N, MW 175.27,



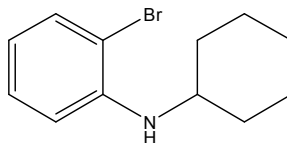
Using the general microwave experimental procedure for ketones the desired product was isolated as a yellow oil, 81 %, LCMS: r.t. 1.79 min (95% MeOH in water), M<sup>+</sup>H: 176.28, HPLC: r.t. 7.14 min (90% acetonitrile in water), 99% pure, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.58-1.90 (6H, m, CH<sub>2</sub>), 2.14-2.18 (2H, m, CH<sub>2</sub>), 2.20 (3H, s, CH<sub>3</sub>), 3.55 (1H, s, NH), 3.90-3.95 (1H, m, CH), 6.70-6.76 (2H, m, ArH), 7.12-7.25 (2H, m, ArH). This compound has been previously synthesised via a different route, by Kuhl *et al.*<sup>145</sup>

**(1-Methylpentyl)-*o*-tolylamine, 89**, C<sub>13</sub>H<sub>21</sub>N, MW 191.31,



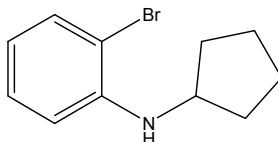
Using the general microwave experimental procedure for ketones the desired product was isolated as an oil, 92 %, LCMS: r.t. 1.78 min (95% MeOH in water), M<sup>+</sup>H: 191.68, HPLC: r.t. 4.25 min (90% acetonitrile in water), 96% pure, IR (CDCl<sub>3</sub>): 2932, 2873, 2256 (CH), 1605, 1510, 1319 (Ar), 1260, 1165 cm<sup>-1</sup> (CO). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.02-1.04 (3H, m, CH<sub>2</sub>CH<sub>3</sub>), 1.30 (3H, d, *J* = 6.2 Hz, CHCH<sub>3</sub>), 1.40-1.75 (6H, m, CH<sub>2</sub>), 2.11 (3H, s, CH<sub>3</sub>), 3.39 (1H, s, NH), 3.56-3.61 (1H, m, CH), 6.68-6.74 (2H, m, ArH), 7.12-7.25 (2H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): δ 14.3 (ArCH<sub>3</sub>), 17.8 (CH<sub>3</sub>CH<sub>2</sub>), 21.2 (CH<sub>3</sub>CH), 23.0, 28.6, 37.2 (CH<sub>2</sub>), 48.4 (CH), 110.1, 116.3 (ArCH), 121.7 (ArC), 127.3, 130.4 (ArCH), 145.7 (ArC).

**Cyclohexyl-*o*-tolylamine, 90**, C<sub>12</sub>H<sub>16</sub>BrN, MW 254.17,



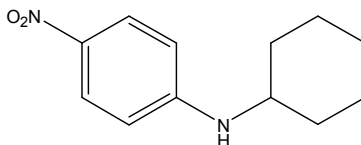
Using the general microwave experimental procedure for ketones the desired product was isolated as an oil, 56 %, LCMS: r.t. 2.15 min (95% MeOH in water), M<sup>+</sup>H 253.64, HPLC: r.t. 2.15 min (90% acetonitrile in water), 91% pure, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.13-1.45 (5H, m, CH<sub>2</sub>), 1.60-1.77 (3H, m, CH<sub>2</sub>), 2.02-2.08 (2H, m, CH<sub>2</sub>), 3.20-3.38 (1H, m, CH), 4.23 (1H, s, NH), 6.52 (1H, td, *J* = 1.5, 7.6 Hz, ArH), 6.65 (1H, dd, *J* = 1.2, 8.2 Hz, ArH), 7.12-7.18 (1H, m, ArH), 7.41 (1H, dd, *J* = 1.5, 6.4 Hz, ArH). This compound has been previously synthesised via a different route, by Basu *et al.*<sup>146</sup>

**(2-Bromophenyl)cyclopentylamine, 91**, C<sub>11</sub>H<sub>14</sub>BrN, MW 240.14,



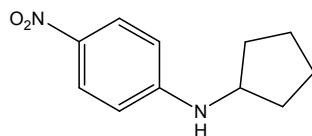
Using the general microwave experimental procedure for ketones the desired product was isolated, 40 %, LCMS: r.t. 2.37 min (95% MeOH in water), M<sup>+</sup>H: 240.30, 242.32, HPLC: r.t. 9.60 min (90% acetonitrile in water), 90% pure, HRMS: Calculated: 240.0382, Measured: 240.0384, IR (CDCl<sub>3</sub>): 3413 (NH), 2962, 2873, 2249 (CH), 1595, 1501, 1325 (Ar), 1019 cm<sup>-1</sup> (CO). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.53-1.78 (6H, m, CH<sub>2</sub>), 1.95-2.15 (2H, m, CH<sub>2</sub>), 3.78-3.83 (1H, m, CH), 4.29 (1H, s, NH), 6.55 (1H, td, *J* = 1.5, 7.4 Hz, ArH), 6.68 (1H, dd, *J* = 1.5, 8.2 Hz, ArH), 7.15-7.22 (1H, m, ArH), 7.41 (1H, dd, *J* = 1.5, 7.9 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz) δ 24.1, 33.6 (CH<sub>2</sub>), 54.7 (CH), 109.8 (ArC), 112.1, 117.6, 128.5, 132.5 (ArCH), 144.8 (ArC).

**Cyclohexyl-(4-nitrophenyl)amine, 92**, C<sub>12</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub>, MW 220.27,



Using the general microwave experimental procedure for ketones the desired product was isolated as a yellow oil, 34 %, LCMS: r.t. 2.64 min (95% MeOH in water), M<sup>+</sup>H: 218.87, HPLC: r.t. 2.35 min (90% acetonitrile in water), 99% pure, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.12-1.45 (5H, m, CH<sub>2</sub>), 1.63-1.80 (3H, m, CH<sub>2</sub>), 1.98-2.05 (2H, m, CH<sub>2</sub>), 3.29-3.40 (1H, m, CH), 4.48-4.51 (1H, m, NH), 6.45-6.50 (2H, m, ArH), 7.98-8.05 (2H, m, ArH). This compound has been previously synthesised using a similar method by Abdel-Magid *et al.*<sup>77</sup>

**Cyclopentyl-(4-nitrophenyl)amine, 93**, C<sub>11</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>, MW 206.24,

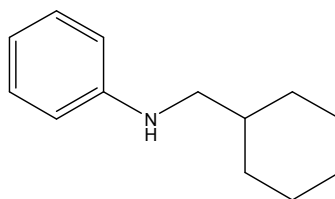


Using the general microwave experimental procedure for ketones the desired product was isolated as an oil, 26 %, LCMS: r.t. 2.19 min (95% MeOH in water), M<sup>+</sup>H: 204.89, HPLC: r.t. 2.14 min (90% acetonitrile in water), 94% pure, IR (CDCl<sub>3</sub>) 3429 (NH), 3085, 2964, 2874, 2252 (CH), 1599, 1518, 1318 (Ar), 1116 cm<sup>-1</sup> (CO), <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.45-1.70 (6H, m, CH<sub>2</sub>), 2.04-2.06 (2H, m, CH<sub>2</sub>), 3.80-3.87 (1H, m, CH), 4.64 (1H, s, NH), 6.47-6.51 (2H, m, ArH), 8.02-8.06 (2H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): δ 24.1, 33.4 (CH<sub>2</sub>), 54.5 (CH), 111.4, 126.5 (ArCH), 137.5, 153.2 (ArC).

#### General Microwave Experimental Procedure for aldehydes<sup>85</sup>

This method is as reported by Bailey *et al.*<sup>85</sup> To a solution of aniline (2 mmol) and aldehyde (1 mmol) in DCE (2 mL) in a microwave vial, was added NaBH(OAc)<sub>3</sub> (2.5 mmol) and AcOH (3 mmol). The vial was capped and the resulting solution was heated in a CEM Discover<sup>®</sup> microwave for 10 minutes (fixed hold time) at 140 °C. The reaction was quenched with a saturated aqueous solution of NaHCO<sub>3</sub> (10 mL) was then extracted with DCM (3 x 15 mL). The combined organics were dried (MgSO<sub>4</sub>), filtered and concentrated *in vacuo*. Purification by flash chromatography (DCM) then proceeded to afford the desired product.

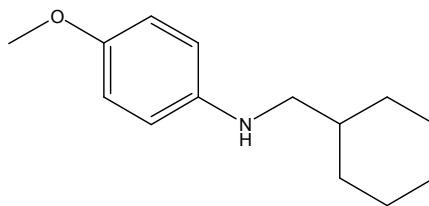
**Cyclohexylmethylphenylamine, 94**, C<sub>13</sub>H<sub>19</sub>N, MW 189.30,



Using the general microwave experimental procedure for aldehydes the desired product was isolated as a cream solid, 91 %, m.p. 82-84 °C (lit. 79-81 °C),<sup>147</sup> LCMS: r.t. 1.80 min (95% MeOH in water), M<sup>+</sup>H: 190.28, HPLC: r.t. 3.46 min (90% acetonitrile in water), 95% pure, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 0.93-1.07 (2H, m, CH<sub>2</sub>), 1.17-1.88 (9H, m, CH<sub>2</sub> and CH), 2.97 (2H, dd, *J* = 2.0, 6.5 Hz, CH<sub>2</sub>NH), 3.72 (1H, s, NH), 6.55-6.72 (3H, m, ArH), 7.15-7.25 (2H, m, ArH).

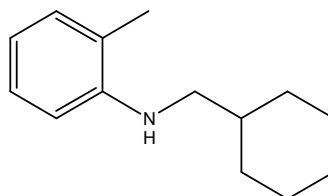


**Cyclohexylmethyl-(4-methoxyphenyl)amine, 95**, C<sub>14</sub>H<sub>21</sub>NO, MW 219.32,



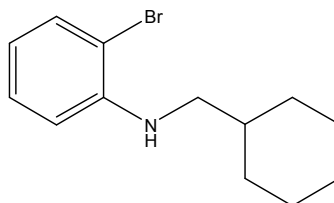
Using the general microwave experimental procedure for aldehydes the desired product was isolated, 83 %, LCMS: r.t. 1.36 min (95% MeOH in water), M<sup>+</sup>H: 220.03, HPLC: r.t. 3.07 min (90% acetonitrile in water), 81% pure, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 0.90-1.05 (2H, m, CH<sub>2</sub>), 1.15-1.24 (3H, m, CH<sub>2</sub> and CH), 1.50-1.85 (6H, m, CH<sub>2</sub>), 2.90 (2H, d, *J* = 6.7 Hz, CH<sub>2</sub>NH), 3.45 (1H, s, NH), 3.75 (3H, s, OCH<sub>3</sub>), 6.55-6.58 (2H, m, ArH), 6.76-6.80 (2H, m, ArH). This compound has been previously synthesised via a different route, by Cho *et al.*<sup>147</sup>

**Cyclohexylmethyl-*o*-tolylamine, 96**, C<sub>14</sub>H<sub>21</sub>N, MW 203.32,



Using the general microwave experimental procedure for aldehydes the desired product was isolated, 88 %, LCMS: r.t. 1.94 min (95% MeOH in water), M<sup>+</sup>H: 203.97, HPLC: r.t. 4.45 min (90% acetonitrile in water), 98% pure, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 0.97-1.40 (5H, m, CH<sub>2</sub> and CH), 1.60-1.85 (6H, m, CH<sub>2</sub>), 2.18 (3H, s, CH<sub>3</sub>), 3.04 (2H, d, *J* = 3.2 Hz, CH<sub>2</sub>NH), 3.59 (1H, s, NH), 6.62-6.67 (2H, m, ArH), 7.08-7.16 (2H, m, ArH). This compound has been previously synthesised via a different route, by Shim *et al.*<sup>148</sup>

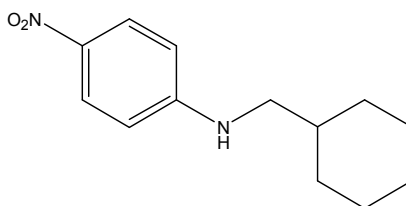
**(2-Bromophenyl)cyclohexylmethylamine, 97**, C<sub>13</sub>H<sub>18</sub>BrN, MW 268.19,



Using the general microwave experimental procedure for aldehydes the desired product was isolated, 75 %, LCMS: r.t. 2.52 min (95% MeOH in water), M<sup>+</sup>H: 270.11, 268.83, HPLC: r.t. 6.22 min (90% acetonitrile in water), 98% pure, IR (CDCl<sub>3</sub>): 3421 (NH), 3073, 2925, 2854 (CH), 1597, 1511, 1324 (Ar), 1019 cm<sup>-1</sup> (CO). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 0.99-1.35 (5H, m, CH<sub>2</sub> and CH), 1.55-1.95 (6H, m, CH<sub>2</sub>), 3.00 (2H, d, *J* = 5.9 Hz, CH<sub>2</sub>NH), 4.40 (1H, s, NH), 6.56-6.65 (2H, m, ArH), 7.10-7.18 (1H, m, ArH), 7.41-

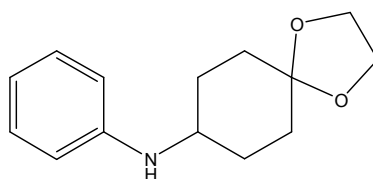
7.53 (1H, m, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz):  $\delta$  26.1, 26.7, 31.4 ( $\text{CH}_2$ ), 37.4 (CH), 50.5 ( $\text{CH}_2\text{NH}$ ), 109.8 (ArC), 111.3, 117.3, 128.6, 132.5 (ArCH), 145.3 (ArC).

**Cyclohexylmethyl-(4-nitrophenyl)amine, 98**,  $\text{C}_{13}\text{H}_{18}\text{N}_2\text{O}_2$ , MW 234.29,



Using the general microwave experimental procedure for aldehydes the desired product was isolated, 61 %, LCMS: r.t. 1.27 min (95% MeOH in water), M-H: 232.99, HPLC: r.t. 2.68 min (90% acetonitrile in water), 99% pure,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  0.90-1.30 (5H, m,  $\text{CH}_2$  and CH), 1.40-1.76 (6H, m,  $\text{CH}_2$ ), 3.00-3.02 (2H, m,  $\text{CH}_2\text{NH}$ ), 4.70 (1H, s, NH), 6.47-6.51 (2H, m, ArH), 8.02-8.06 (2H, m, ArH). This compound was previously synthesised using a similar method by Abdel-Magid *et al.*<sup>77</sup>

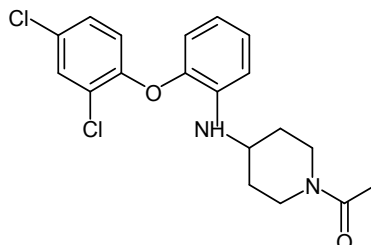
**(1,4-Dioxaspiro[4.5]dec-8-yl)phenylamine, 99**,  $\text{C}_{14}\text{H}_{19}\text{NO}_2$ , MW 233.31,



Using the general microwave experimental procedure for ketones the desired product was isolated as an off white solid, 82 %, m.p. 113-115 °C (lit. 112-113 °C),<sup>149</sup> LCMS: r.t. 1.25 min (95% MeOH in water),  $\text{M}^+\text{H}$ : 233.93, HPLC: r.t. 2.28 min (90% acetonitrile in water), 95% pure,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.42-1.82 (6H, m,  $\text{CH}_2$ ), 2.01-2.10 (2H, m,  $\text{CH}_2$ ), 3.29-3.40 (1H, s, CH), 3.52 (1H, s, NH), 3.94 (4H, m,  $\text{CH}_2\text{O}$ ), 6.57-6.70 (3H, m, ArH), 7.13-7.16 (2H, m, ArH).

**1-(4-[2-(2, 4-Chloro-phenoxy)-phenylamino]-piperidin-1-yl)-ethanone, 100**,

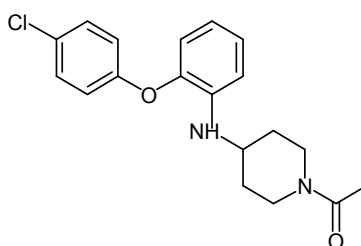
$\text{C}_{19}\text{H}_{20}\text{Cl}_2\text{N}_2\text{O}_2$ , MW 379.29,



To a solution of 4-[2-(2,4-dichloro-phenoxy)-phenylcarbamoyl]-piperidine-1-carboxylic acid tert-butyl ester (100 mg, 0.45 mmol), 1-acetyl-4-piperidone (0.12 mL, 0.9 mmol) and AcOH (0.24 mL) in DCE (2 mL) was added sodium triacetoxyborohydride (0.26 g, 1.1 mmol). The solution was heated using a CEM microwave at 100 °C for 15 min. The mixture was allowed to cool and sat.  $\text{NaHCO}_3$  was added (10 mL) and extracted

with EtOAc (2 x 20 mL). The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % EtOAc in hexane) to afford the title compound as a white solid, 148 mg, 99 % yield. R.f: 0.55 (EtOAc), mp. 144-145 °C, LCMS *t*<sub>r</sub> = 5.40 min (50 % MeOH in water at 0.5 mL/min), *m/z* M+H 379.41, HPLC *t*<sub>r</sub> = 2.65 min (90% acetonitrile in water at 1.0 mL/min), 99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.27-1.43 (2H, m, CH<sub>2</sub>), 2.4-2.11 (5H, m, CH<sub>3</sub>, CH<sub>2</sub>), 2.84-2.94 (1H, m, CH<sub>2</sub>), 3.15-3.25 (1H, m, CH<sub>2</sub>), 3.42-3.55 (1H, m, CH), 3.74-3.78 (1H, m, CH<sub>2</sub>), 4.06-4.08 (1H, m, NH), 4.35-4.43 (1H, m, CH<sub>2</sub>), 6.60-6.66 (1H, m, ArH), 6.72-6.77 (2H, m, ArH), 6.80 (1H, d, *J* = 2.5 Hz, ArH), 7.01-7.09 (1H, m, ArH), 7.13 (1H, dd, *J* = 8.9, 2.5 Hz, ArH), 7.44 (1H, d, *J* = 2.5 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 21.4 (CH<sub>3</sub>), 31.8, 32.6, 40.1, 44.9 (CH<sub>2</sub>), 49.5 (CH), 112.3, 117.0, 118.6, 119.5, 125.3, 127.9 (ArCH), 128.5 (ArC), 130.3 (ArCH), 138.2, 142.9, 151.4 (ArC), 168.8 (CO). Anal. Calcd for C<sub>19</sub>H<sub>21</sub>ClN<sub>2</sub>O<sub>2</sub>: C 60.17, H 5.32, N 7.39 %, Found C 60.2, H 5.57, N 7.22.

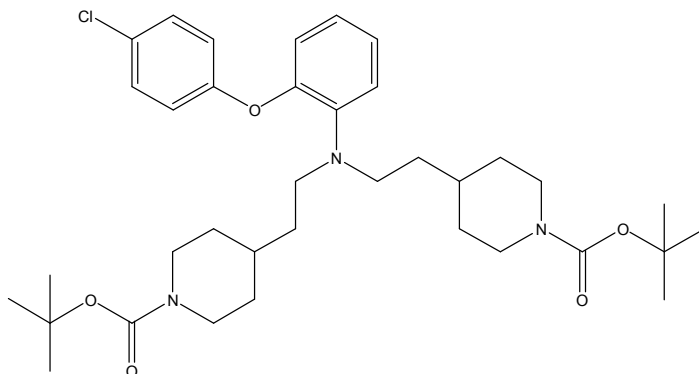
**1-(4-[2-(4-Chloro-phenoxy)-phenylamino]-piperidin-1-yl)-ethanone, 101,**  
C<sub>19</sub>H<sub>21</sub>ClN<sub>2</sub>O<sub>2</sub>, MW 344.86,



To a solution of 4-[2-(4-chloro-phenoxy)-phenylcarbamoyl]-piperidine-1-carboxylic acid tert-butyl ester (100 mg, 0.45 mmol), 1-acetyl-4-piperidone (0.12 mL, 0.9 mmol) and AcOH (0.24 mL) in DCE (2 mL) was added sodium triacetoxyborohydride (0.26 g, 1.13 mmol). The solution was heated using a CEM microwave at 100 °C for 15 min. The mixture was allowed to cool and sat. NaHCO<sub>3</sub> was added (10 mL) and the mixture was extracted with EtOAc (2 x 20 mL). The combined organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % EtOAc in hexane) to afford the title compound as a white solid, 153 mg, 98 % yield. mp. 129-130 °C, R.f: 0.41 (DCM), LCMS: *t*<sub>r</sub> = 5.05 min (50 % MeOH in water at 0.5 mL/min), *m/z* M+H 345.40, HPLC: *t*<sub>r</sub> = 2.45 min (90 % acetonitrile in water at 1.0 mL/min), >99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.23-1.37 (2H, m, CH<sub>2</sub>), 2.01-2.14 (5H, m, CH<sub>2</sub>, CH<sub>3</sub>), 2.80-2.90 (1H, m, CH<sub>2</sub>), 3.13-3.23 (1H, m, CH<sub>2</sub>), 3.48-3.52 (1H, m, CH), 3.72-3.78 (1H, m, CH<sub>2</sub>), 4.00 (1H, s, NH), 4.37-4.42 (1H, m, CH<sub>2</sub>), 6.64 (1H, td, *J* = 6.4, 1.2 Hz, ArH), 6.74 (1H, dd, *J* = 8.2, 1.5 Hz, ArH), 6.80 (1H, dd, *J* = 6.4, 1.5 Hz, ArH), 6.84-6.88 (2H, m, ArH), 7.03 (1H, td, *J* = 7.4, 1.5 Hz, ArH), 7.21-7.27 (2H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 21.4 (CH<sub>3</sub>), 31.9, 32.7, 40.2, 45.0 (CH<sub>2</sub>), 49.6 (CH), 112.2, 117.2, 118.6, 119.5, 125.2 (ArCH), 127.8 (ArC),

129.7 (ArCH), 138.7, 142.9, 156.0 (ArC), 168.8 (CO). Anal. Calcd for  $C_{19}H_{21}ClN_2O_2$ : C 66.18, H 6.14, N 8.12. Found C 66.0, H 6.06, N 7.90.

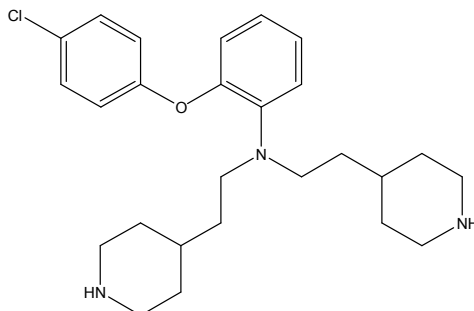
**4-(2-[2-(4-Chloro-phenoxy)-phenylamino]-di-ethyl)-di-piperidine-1-carboxylic acid tert-butyl ester, 106**,  $C_{36}H_{52}ClN_3O_5$ , MW 642.27,



A mixture of 2-(4-chloro-phenoxy)-phenylamine (200 mg, 0.91 mmol), *N*-Boc-4-piperidine acetaldehyde (413 mg, 1.8 mmol),  $NaBH(OAc)_3$  (483 mg, 2.3 mmol) and AcOH (0.16 mL) in DCE (3 mL) was stirred at r.t. for 1 h.  $NaHCO_3$  (20 mL) was added and the mixture was extracted with DCM (2 x 20 mL). The crude product was purified by flash chromatography (0-30 % EtOAc in DCM) to yield the desired product as a cream oil, 584 mg, 74 % yield. R.f. 0.35 (DCM), LCMS:  $t_r$  = 5.93 min (90 % MeOH in water),  $m/z$   $M+Na$  663.6, HPLC:  $t_r$  = 3.5 min (100 % MeOH), 99 %,  $^1H$  NMR ( $CDCl_3$ , 270 MHz):  $\delta$  0.98-1.05 (4H, m,  $2CH_2$ ), 1.25-1.33 (4H, m,  $2CH_2$ ), 1.42 (9H, s,  $tBu$ ), 1.43-1.53 (2H, m,  $2CH$ ), 2.55 (4H, t,  $J$  = 11.8 Hz,  $2CH_2$ ), 3.07 (4H, t,  $J$  = 6.9 Hz,  $2CH_2$ ), 3.99 (4H, m,  $2CH_2$ ), 6.71-6.79 (2H, m, ArH), 6.91-7.55 (6H, m, ArH).  $^{13}C$  NMR ( $CDCl_3$ , 68 MHz): 28.5 ( $tBu$ ), 32.2, 33.6 ( $CH_2$ ), 33.9 (CH), 49.6, 62.0 ( $CH_2$ ), 117.9, 121.7, 122.4, 122.4, 125.1 (ArCH), 126.9 (ArC), 129.4 (ArCH), 142.9, 148.7, 154.9 (ArC). HRMS: Calcd for  $C_{36}H_{52}ClN_3O_5$  ( $M+H$ ) $^+$  642.3668, found ( $M+H$ ) $^+$  642.3679.

**[2-(4-Chloro-phenoxy)-phenyl]-bis-(2-piperidin-4-yl-ethyl)-amine, 107**,

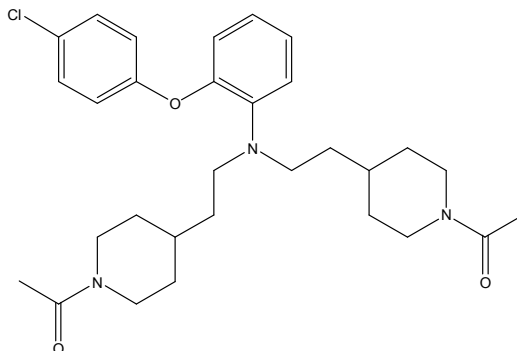
$C_{26}H_{36}ClN_3O$ , MW 442.04,



Using the general procedure for deprotection the desired product was obtained as a cream oil, 170 mg, 71 % yield. R.f. 0.30 (EtOAc),  $^1H$  NMR ( $CDCl_3$ , 270 MHz):  $\delta$  1.00-1.10 (4H, m,  $2CH_2$ ), 1.18-1.32 (6H, m,  $2CH$  and  $2CH_2$ ), 1.55 (4H, d,  $J$  = 12.8 Hz,

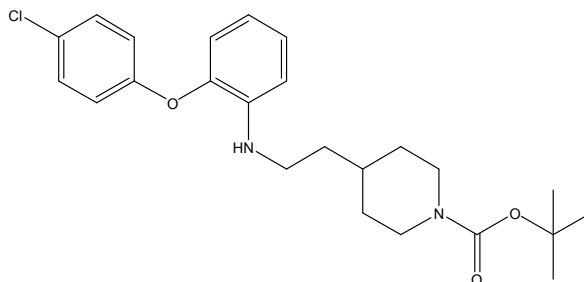
2CH<sub>2</sub>), 2.47 (4H, t,  $J$  = 12.0 Hz, 2CH<sub>2</sub>), 2.81 (2H, br.s 2NH), 3.00 (4H, d,  $J$  = 8.1 Hz, 2CH<sub>2</sub>), 3.07 (4H, t,  $J$  = 7.6 Hz, 2CH<sub>2</sub>), 6.73-6.77 (2H, m, ArH), 6.91-6.94 (2H, m, ArH), 6.99 (1H, d,  $J$  = 8.0 Hz, ArH), 7.07-7.11 (1H, m, ArH), 7.16-7.20 (2H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): 33.0 (CH<sub>2</sub>), 33.9 (CH), 34.0, 46.3, 49.4 (CH<sub>2</sub>), 117.8, 121.6, 122.1, 122.3, 125.0 (ArCH), 126.7 (ArC), 129.2 (ArCH), 143.0, 148.5, 156.8 (ArC).

**1-[4-(2-([2-(1-Acetyl-piperidin-4-yl)-ethyl]-[2-(4-chloro-phenoxy)-phenyl]-amino)-ethyl)-piperidin-1-yl]-ethanone, 108**, C<sub>30</sub>H<sub>40</sub>ClN<sub>3</sub>O<sub>3</sub>, MW 526.11,



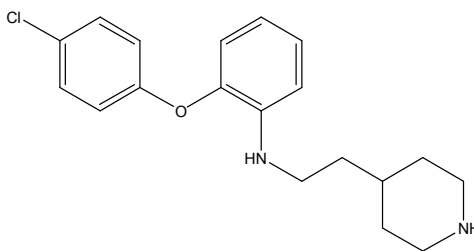
[2-(4-chloro-phenoxy)-phenyl]-bis-(2-piperidin-4-yl-ethyl)-amine (97 mg, 0.22 mmol) was dissolved in DCM (5 mL) and cooled to 0 °C, to this was added TEA (0.06 mL, 0.88 mmol) and acetyl chloride (0.1 mL, 1.32 mmol). The resulting solution was stirred at r.t. for 1 h. NaHCO<sub>3</sub> (20 mL) was added and the mixture was extracted with DCM (2 x 20 mL). The crude material was purified by flash chromatography (0-95 % EtOAc in hexane) to yield the desired product as a clear oil, 83 mg, 72 % yield. R.f. 0.65 (EtOAc), LCMS:  $t_r$  = 1.46 min (90 % MeOH in water),  $m/z$  M+H 526.00, HPLC:  $t_r$  = 2.35 min (90 % acetonitrile in water), 98 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  0.95-1.04 (4H, m, 2CH<sub>2</sub>), 1.28-1.34 (6H, m, 2CH and 2CH<sub>2</sub>), 1.57 (4H, d,  $J$  = 12.8 Hz, 2CH<sub>2</sub>), 2.03 (6H, s, 2CH<sub>3</sub>), 2.38 (2H, td,  $J$  = 2.8, 13.2 Hz, 2x ½CH<sub>2</sub>), 2.86 (2H, td,  $J$  = 2.4, 13.2 Hz, 2x ½CH<sub>2</sub>), 3.05-3.11 (4H, m, 2CH<sub>2</sub>), 3.80 (2H, d,  $J$  = 12.0 Hz, 2x ½CH<sub>2</sub>), 4.48-4.52 (2H, m, 2x ½CH<sub>2</sub>), 6.72-6.76 (2H, m, ArH), 6.92-6.94 (2H, m, ArH), 6.95-7.00 (1H, m, ArH), 7.08-7.12 (1H, m, ArH), 7.16-7.20 (2H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): 21.4 (CH<sub>3</sub>), 31.8, 32.7, 33.3 (CH<sub>2</sub>), 33.8 (CH), 41.7, 46.5, 49.4 (CH<sub>2</sub>), 117.7, 121.7, 122.4, 122.5, 125.1 (ArCH), 126.8 (ArC), 129.3 (ArCH), 142.7, 148.6, 156.7 (ArC), 168.7 (CO). HRMS: Calcd for C<sub>30</sub>H<sub>40</sub>ClN<sub>3</sub>O<sub>3</sub> (M+H)<sup>+</sup> 526.2831, found (M+H)<sup>+</sup> 526.2840.

**4-(2-[2-(4-Chloro-phenoxy)-phenylamino]-ethyl)-piperidine-1-carboxylic acid tert-butyl ester, 109**, C<sub>24</sub>H<sub>31</sub>ClN<sub>2</sub>O<sub>3</sub>, MW 430.97,



A mixture of 2-(4-chloro-phenoxy)-phenylamine (400 mg, 1.8 mmol), *N*-Boc-4-piperidine acetaldehyde (210 mg, 0.91 mmol), NaBH(OAc)<sub>3</sub> (483 mg, 2.3 mmol) and AcOH (0.16 mL) in DCE (3 mL) was stirred at r.t. for 1 h. NaHCO<sub>3</sub> (20 mL) was added and the mixture was extracted with DCM (2 x 20 mL). The crude product was purified by flash chromatography (0-100 % DCM in hexane) to yield the desired product as a colourless oil, 383 mg, 96 % yield. R.f. 0.30 (DCM), LCMS: *t*<sub>r</sub> = 2.8 min (90 % MeOH in water), *m/z* M+Na 453.0, HPLC: *t*<sub>r</sub> = 2.47 min (90 % acetonitrile in water), 93 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 1.05-1.14 (2H, m, CH<sub>2</sub>), 1.45 (9H, s, (CH<sub>3</sub>)<sub>3</sub>), 1.46-1.57 (3H, m, CH<sub>2</sub> and CH), 1.62 (2H, d, *J*=12.4Hz, CH<sub>2</sub>), 2.61-2.68 (2H, m, CH<sub>2</sub>), 3.16 (2H, t, *J*=7.2Hz, CH<sub>2</sub>), 3.96 (1H, br.s, NH), 4.05 (2H, d, *J*=12.4Hz, CH<sub>2</sub>), 6.63 (1H, td, *J*= 0.8, 7.2 Hz, ArH), 6.71 (1H, dd, *J*=1.6, 8.4Hz, ArH), 6.81 (1H, dd, *J*=1.2, 8.0 Hz, ArH), 6.87-6.89 (2H, m, ArH), 7.05 (1H, td, *J*= 1.2, 7.6Hz, ArH), 7.23-7.25 (2H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): 28.5 ((CH<sub>3</sub>)<sub>3</sub>), 32.1 (CH<sub>2</sub>), 33.9 (CH), 36.1, 41.0 (CH<sub>2</sub>), 79.3 (C(CH<sub>3</sub>)<sub>3</sub>), 111.5, 116.7, 118.5, 119.5, 125.4 (ArCH), 127.7 (ArC), 129.6 (ArCH), 140.6, 142.7, 154.9 (ArC), 156.3 (CO). HRMS: Calcd for C<sub>24</sub>H<sub>31</sub>ClN<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 431.2096, found (M+H)<sup>+</sup> 431.2113.

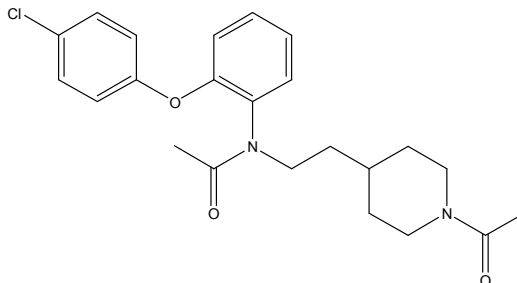
**[2-(4-Chloro-phenoxy)-phenyl]-(2-piperidin-4-yl-ethyl)-amine, 110**, C<sub>19</sub>H<sub>23</sub>ClN<sub>2</sub>O, MW 330.85,



Using the general procedure for deprotection the desired product was obtained as a cream oil, 209 mg, 97 % yield. R.f. 0.32 (EtOAc), HPLC: *t*<sub>r</sub> = 1.55 min (90 % acetonitrile in water), 80 %. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 1.28-1.36 (2H, m, CH<sub>2</sub>), 1.49-1.59 (3H, m, CH<sub>2</sub> and CH), 1.75 (2H, d, *J*= 13.2Hz, CH<sub>2</sub>), 2.66 (2H, td, *J*= 2.8, 12.8Hz, CH<sub>2</sub>), 3.13-3.21 (4H, m, CH<sub>2</sub>), 3.95 (1H, t, *J*= 5.2Hz, NHCH<sub>2</sub>), 5.97 (1H, br.s, NH), 6.64 (1H, td, *J*= 1.2, 7.2Hz, ArH), 6.67-6.71 (1H, m, ArH), 6.81 (1H, dd, *J*= 7.6,

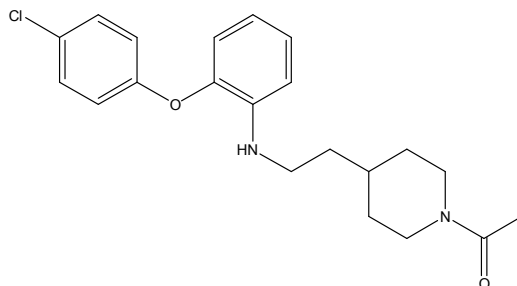
0.8Hz, ArH), 6.86-6.90 (1H, m, ArH), 7.05 (1H, td,  $J = 1.6$ , 8.0Hz, ArH), 7.12-7.14 (1H, m, ArH), 7.22-7.26 (2H, m, ArH).

***N*-[2-(1-Acetyl-piperidin-4-yl)-ethyl]-*N*-[2-(4-chloro-phenoxy)-phenyl]-acetamide, 111**, C<sub>23</sub>H<sub>27</sub>ClN<sub>2</sub>O<sub>3</sub>, MW 414.93,



[2-(4-Chloro-phenoxy)-phenyl]-(2-piperidin-4-yl-ethyl)-amine (114 mg, 0.35 mmol) was dissolved in DCM (5 mL) and cooled to 0 °C, to this was added TEA (0.06 mL, 0.88 mmol) and acetyl chloride (0.03 mL, 1.05 mmol). The resulting solution was stirred at r.t. for 1 h. NaHCO<sub>3</sub> (20 mL) was added and the mixture was extracted with DCM (2 x 20 mL). The crude material was purified by flash chromatography (0-95 % EtOAc in hexane) to yield the desired product as a clear oil, 93 mg, 65 % yield. R.f. 0.48 (EtOAc), LCMS:  $t_r = 1.45$  min (90 % MeOH in water),  $m/z$  M+H 415.00, HPLC:  $t_r = 1.81$  min (90 % acetonitrile in water), 95 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  0.97-1.11(2H, m, CH<sub>2</sub>), 1.38-1.57 (3H, m, CH and CH<sub>2</sub>), 1.66-1.78 (2H, m, CH<sub>2</sub>), 1.85 (3H, s, CH<sub>3</sub>), 2.03 (3H, s, CH<sub>3</sub>), 2.38-2.51 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 2.89-2.99 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 3.51-3.58 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 3.69-3.86 (2H, m, 2x  $\frac{1}{2}$ CH<sub>2</sub>), 4.47-4.55 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 6.88-6.96 (3H, m, ArH), 7.13-7.20 (2H, m, ArH), 7.27-7.32 (3H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): 21.5, 22.3 (CH<sub>3</sub>), 31.8, 31.8, 32.4, 32.5 (cyclic CH<sub>2</sub>), 33.7 (CH<sub>2</sub>), 34.2, 34.3 (cyclic CH<sub>2</sub>), 41.7 (CH<sub>2</sub>), 45.8, 45.9 (cyclic CH<sub>2</sub>), 46.6 (CH<sub>2</sub>), 119.1, 120.0, 124.5, 129.4, 130.0, 130.4 (ArCH), 130.4, 133.4, 152.9, 154.6 (ArC), 168.7, (170.7, 170.7) (CO). HRMS: Calcd for C<sub>23</sub>H<sub>27</sub>ClN<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 415.1783, found (M+H)<sup>+</sup> 415.1778.

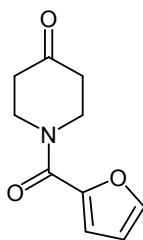
**1-(4-(2-[2-(4-Chloro-phenoxy)-phenylamino]-ethyl)-piperidin-1-yl)-ethanone, 112**, C<sub>21</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>2</sub>, MW 372.89,



[2-(4-Chloro-phenoxy)-phenyl]-(2-piperidin-4-yl-ethyl)-amine (50 mg, 0.15 mmol) was dissolved in DCM (2 mL) and cooled to -10 °C, to this was added TEA (0.021 mL, 0.15 mmol) and acetyl chloride (0.011 mL, 0.15 mmol). The resulting solution was stirred at

-10 °C for 10 min. NaHCO<sub>3</sub> (20 mL) was added and the mixture was extracted with DCM (2 x 20 mL). The crude material was purified by flash chromatography (0-10 % MeOH in DCM) to yield the desired product as an off white oil, 31 mg, 55 % yield. R.f. 0.55 (EtOAc), LCMS:  $t_r$  = 2.60 min (90 % MeOH in water),  $m/z$  M-H 371.4, HPLC:  $t_r$  = 4.58 min (90 % acetonitrile in water), 96 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  1.07-1.18 (2H, m, CH<sub>2</sub>), 1.51-1.59 (3H, m, CH<sub>2</sub> and CH), 1.69-1.72 (2H, m, CH<sub>2</sub>), 2.07 (3H, s, CH<sub>3</sub>), 2.48 (1H, td,  $J$  = 3.2, 12.8 Hz,  $\frac{1}{2}$ CH<sub>2</sub>), 2.97 (1H, td,  $J$  = 2.8, 13.2 Hz,  $\frac{1}{2}$ CH<sub>2</sub>), 3.17 (2H, t,  $J$  = 6.8 Hz,  $\frac{1}{2}$ CH<sub>2</sub>), 3.73-3.78 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 3.97 (1H, br.s, NH), 4.55-4.61 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 6.65 (1H, td,  $J$  = 1.6, 7.6 Hz, ArH), 6.71 (1H, dd,  $J$  = 1.2, 7.6 Hz, ArH), 6.82 (1H, dd,  $J$  = 1.6, 8.4 Hz, ArH), 6.86-6.90 (2H, m, ArH), 7.06 (1H, td,  $J$  = 1.2, 8.0 Hz, ArH), 7.23-7.32 (2H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): 21.5 (CH<sub>3</sub>), 31.7, 32.7 (CH<sub>2</sub>), 33.8 (CH), 35.8, 40.8, 41.6, 46.5 (CH<sub>2</sub>), 111.4, 116.7, 118.4, 119.4, 125.4 (ArCH), 127.6 (ArC), 129.6 (ArCH), 129.6 (ArC), 129.6 (ArCH), 140.3, 142.5, 156.2 (ArC), 168.7 (CO). HRMS: Calcd for C<sub>21</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup> 373.1677, found (M+H)<sup>+</sup> 373.1663.

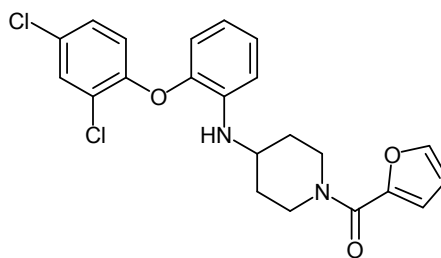
**1-(Furan-2-carbonyl)-piperidin-4-one, 113**, C<sub>10</sub>H<sub>11</sub>NO<sub>3</sub>, MW 193.07,



To a solution of 4-piperidone hydrochloride monohydrate (230 mg, 1.5 mmol) in DCM (5 mL) was added MP-Carbonate resin (0.58 g). The mixture was stirred at room temperature for 2 h. In a separate flask, 2-furoic acid (200 mg, 1.8 mmol), *N,N*-4-dimethylaminopyridine (cat.), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.85 g, 4.44 mmol) and TEA (0.15 mL) were dissolved in DCM (5 mL) and stirred at room temperature for 2 h. The two reaction mixtures were then combined and stirred at room temperature for a further 18 h. The resulting solution was then filtered to remove the resin. HCl (1M, 20 mL) was added and the mixture was extracted. The organic layers were washed with NaHCO<sub>3</sub> (20 mL) and then brine (20 mL). The organic layers was dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*, to yield the title compound as a yellow oil, 250 mg, 86 % yield. LCMS:  $t_r$  = 1.99 min (50 % MeOH in water at 0.5 mL/min),  $m/z$  M+H 194.16, HPLC:  $t_r$  = 1.85 min (90 % acetonitrile in water at 1.0 mL/min), 97 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  2.53 (4H, t,  $J$  = 6.5 Hz, CH<sub>2</sub>), 4.03 (4H, s, CH<sub>2</sub>), 9.49 (1H, q,  $J$  = 1.7 Hz, ArH), 7.07 (1H, dd,  $J$  = 0.7, 3.5 Hz, ArH), 7.48 (1H, q,  $J$  = 1.0 Hz, ArH).

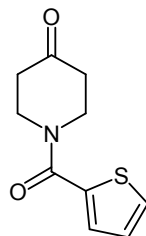


**4-[2-(2, 4-Dichloro-phenoxy)-phenylamino]-piperidin-1-yl-furan-2-yl-methanone, 114**, C<sub>22</sub>H<sub>20</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>, MW 431.31,



To a solution of 2-(2,4-dichloro-phenoxy)-phenylamine (100 mg, 0.39 mmol), 1-(furan-2-carbonyl)-piperidin-4-one (91 mg, 0.47 mmol) and sodium triacetoxyborohydride (116 mg, 0.55 mmol) in DCE, was added AcOH (0.1 mL). The resulting reaction mixture was stirred at r.t. for 4 days. NaHCO<sub>3</sub> (20 mL) was then added and the solution was extracted with ethyl acetate (3 x 20 mL). The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated to *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to afford the desired product as a yellow oil, 25 mg, 15 % yield. R.f. 0.7 (5 % Methanol–DCM), LCMS: *t*<sub>r</sub> = 5.66 min (50 % MeOH in water at 0.5 mL/min), *m/z* M+H 431.41, HPLC: *t*<sub>r</sub> = 2.99 min (90% acetonitrile in water at 1.0 mL/min), 99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.39-1.54 (2H, m, CH<sub>2</sub>), 2.13 (2H, dd, *J* = 3.6, 12.8 Hz, CH<sub>2</sub>), 3.21 (2H, s, CH<sub>2</sub>) 3.58-3.65 (1H, m, CH<sub>2</sub>), 4.11 (1H, s, NH), 4.38 (2H, d, *J* = 12.8 Hz, CH<sub>2</sub>), 6.46 (1H, dd, *J* = 1.6, 3.6 Hz, ArH), 6.64 (1H, td, *J* = 1.6, 7.6 Hz, ArH), 6.74 (1H, dd, *J* = 1.6, 8.0 Hz, ArH), 6.78 (1H, dd, *J* = 1.2, 8.4 Hz, ArH), 6.81 (1H, d, *J* = 8.8 Hz, ArH), 6.96 (1H, d, *J* = 3.2 Hz, ArH), 7.05 (1H, td, *J* = 1.2, 8.0 Hz, ArH), 7.13 (1H, dd, *J* = 2.4, 8.8 Hz, ArH), 7.44 (1H, d, *J* = 2.4 Hz, ArH), 7.46 (1H, d, *J* = 2.0 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 49.69 (CH), 112.21, 112.29, 116.15, 116.99, 118.63, 119.48, 125.31, 127.94, 128.55, 130.30, 138.25, 142.89, 143.59, 147.89 (ArC), 151.47, 159.16 (C=O). HRMS: Calcd for C<sub>22</sub>H<sub>20</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 431.0924, found (M+H)<sup>+</sup> 431.0925.

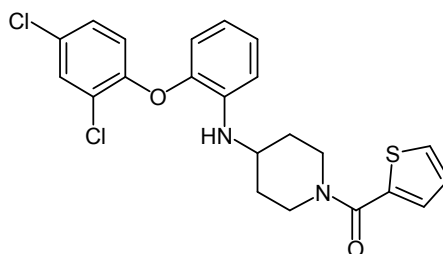
**1-(Thiophene-2-carbonyl)-piperidin-4-one, 115**, C<sub>10</sub>H<sub>11</sub>NO<sub>2</sub>S, MW 209.26,



To a solution of 4-piperidone hydrochloride monohydrate (320 mg, 2.1 mmol) in DCM (7 mL) was added MP-Carbonate resin (0.8 g). This was stirred at room temperature for 2 h. In a separate flask, 2-thiophenecarboxylic acid (320 mg, 2.5 mmol), *N,N*-4-dimethylaminopyridine (cat.), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (1.2 g, 6.3 mmol) and TEA (0.25 mL) were dissolved in DCM (7 mL) and stirred at room temperature for 2 h. The two reaction mixtures were then combined

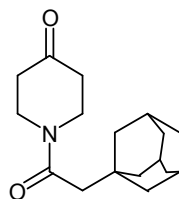
and stirred at room temperature for a further 18 h. The resulting solution was then filtered to remove the resin. HCl (1M, 20 mL) was then added and the mixture was extracted. The organic layers were then washed with NaHCO<sub>3</sub> (20 mL) and then brine (20 mL). The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to yield the desired product as a white solid, 220 mg, 56 % yield. m.p. 81-83 °C, R<sub>f</sub> 0.5 (1:9, MeOH: DCM), LCMS: *t*<sub>r</sub> = 2.29 min (50 % MeOH in water at 0.5 mL/min), *m/z* M+H 210.11, HPLC: *t*<sub>r</sub> = 1.89 min (90 % acetonitrile in water at 1.0 mL/min), >99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 2.47 (4H, t, *J* = 6.2 Hz, CH<sub>2</sub>), 3.94 (4H, t, *J* = 6.4 Hz, CH<sub>2</sub>), 7.00 (1H, dd, *J* = 3.5, 4.9 Hz, ArH), 7.31 (1H, dd, *J* = 1.2, 3.7 Hz, ArH), 7.43 (1H, dd, *J* = 1.2, 4.9 Hz, ArH),

**4-[2-(2,4-Dichloro-phenoxy)-phenylamino]-piperidin-1-yl-thiophen-2-yl-methanone, 116**, C<sub>22</sub>H<sub>20</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>S, MW 447.38



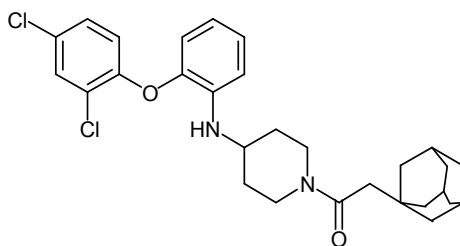
To a solution of 2-(2,4-dichloro-phenoxy)-phenylamine (185 mg, 0.72 mmol) in DCE (5 mL) was added 1-(thiophene-2-carbonyl)-piperidin-4-one (150 mg, 0.72 mmol), sodium triacetoxyborohydride (230 mg, 1.08 mmol) and AcOH (0.21 mmol, 3.6 mmol). The resulting reaction mixture was stirred at r.t. for 7 days and the reaction was monitored by TLC. NaHCO<sub>3</sub> (20 mL) was then added and extracted with EtOAc (3 x 25 mL). The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to afford the title compound as a light brown oil, 162 mg, 50 % yield. m.p. 128-129 °C, LCMS: *t*<sub>r</sub> = 5.7 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min), *m/z* M+H 447.34, HPLC: *t*<sub>r</sub> = 4.03 min (90% acetonitrile in water at 0.8 mL/min), >99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz, 50 °C): δ 1.42-1.52 (2H, m, CH<sub>2</sub>), 2.11 (2H, dd, *J* = 13.2, 3.2 Hz, CH<sub>2</sub>), 3.22 (2H, t, *J* = 11.6 Hz, CH<sub>2</sub>), 3.61 (1H, s, CH), 4.12 (1H, s, NH), 4.29 (2H, s, CH<sub>2</sub>), 6.64 (1H, td, *J* = 1.6, 7.2 Hz, ArH), 6.74 (1H, dd, *J* = 1.6, 7.6 Hz, ArH), 6.77 (1H, dd, *J* = 1.6, 8.0 Hz, ArH), 6.81 (1H, d, *J* = 8.4 Hz, ArH), 7.02 (1H, dd, *J* = 3.6, 5.2 Hz, ArH), 7.04 (1H, td, *J* = 7.6, 1.6 Hz, ArH), 7.13 (1H, dd, *J* = 2.0, 8.4 Hz, ArH), 7.26 (1H, dd, *J* = 1.2, 3.6 Hz, ArH), 7.42 (1H, dd, *J* = 1.2, 5.2 Hz, ArH), 7.44 (1H, d, *J* = 2.4 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 29.59, 32.38 (CH<sub>2</sub>), 49.57 (CH), 112.23, 116.98, 118.54, 119.48, 125.25, 126.58, 127.89, 128.42, 128.49, 128.54, 130.23 (ArCH), 137.00, 138.14, 142.84, 151.40 (ArC), 163.47 (C=O).

**1-(2-Adamantan-1-yl-acetyl)-piperidin-4-one, 117**, C<sub>17</sub>H<sub>25</sub>NO<sub>2</sub>, MW 275.39,



To a solution of 4-piperidone hydrochloride monohydrate (320 mg, 2.1 mmol) in DCM (7 mL) was added MP-Carbonate resin (0.8 g). This was stirred at room temperature for 2 h. In a separate flask, 1-adamantane acetic acid (490 mg, 2.5 mmol), *N,N*-4-dimethylaminopyridine (cat.), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.2 g, 6.3 mmol) and TEA (0.25 mL) were dissolved in DCM (7 mL) and stirred at room temperature for 2 h. The two reaction mixtures were then combined and stirred at room temperature for a further 18 h. The resulting solution was then filtered to remove the resin. HCl (1M, 20 mL) was added to the filtrate, which was extracted with DCM (3 x 20 mL). The organic portions were washed with NaHCO<sub>3</sub> (20 mL) then brine (20 mL). The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to yield a white solid, 310 mg, 60 % yield. m.p. 114-115 °C, LCMS: *t<sub>r</sub>* = 4.68 min (50 % to 95 % MeOH in water at 0.5 mL/min), *m/z* M+H 276.44, HPLC: *t<sub>r</sub>* = 2.56 min (90 % acetonitrile in water at 1.0 mL/min), 99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.57-1.67 (12H, m, CH<sub>2</sub>), 1.96 (3H, s, CH), 2.22 (2H, s, CH<sub>2</sub>CO), 2.46 (4H, t, *J* = 6.2 Hz, CH<sub>2</sub>CH<sub>2</sub>N), 3.80 (2H, t, *J* = 6.2 Hz, CH<sub>2</sub>N), 3.89 (2H, t, *J* = 6.2 Hz, CH<sub>2</sub>N).

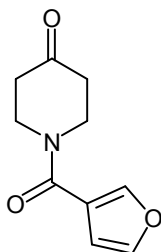
**2-Adamantan-1-yl-1-(4-[2-(2,4-dichloro-phenoxy)-phenylamino]-piperidin-1-yl)-ethanone, 118**, C<sub>29</sub>H<sub>34</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>, MW 513.50,



A solution of 2-(2,4-dichloro-phenoxy)-phenylamine (140 mg, 0.55 mmol), 1-(2-adamantan-1-yl-acetyl)-piperidin-4-one (150 mg, 0.55 mmol), sodium triacetoxyborohydride (175 mg, 0.83 mmol) and AcOH (0.16 mmol, 2.75 mmol) in DCE (5 mL) was stirred at r.t. for 7 days. NaHCO<sub>3</sub> (20 mL) was then added and the mixture was extracted with EtOAc (3 x 30 mL). The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to afford the desired compound as a brown oil, 100 mg, 35 % yield. LCMS: *t<sub>r</sub>* = 3.6 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min), *m/z* M+H 513.50, HPLC: *t<sub>r</sub>* = 9.94 min (90 %

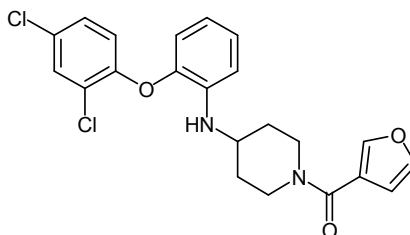
acetonitrile in water at 0.8 mL/min), 98 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.29-1.41 (2H, m,  $\text{CH}_2$ ), 1.61-1.70 (12H, m,  $6\text{CH}_2$ ), 1.95 (3H, s, CH), 2.04-2.07 (2H, m,  $\text{CH}_2$ ), 2.14 (2H, d,  $J = 1.2$  Hz,  $\text{CH}_2\text{CO}$ ), 2.88 (1H, ddd,  $J = 3.2, 11.2, 14.0$  Hz,  $\text{CH}_2$ ), 3.19 (1H, ddd,  $J = 2.8, 11.2, 14.0$  Hz,  $\text{CH}_2$ ), 3.50-3.55 (1H, m, CH), 3.86 (1H, d,  $J = 12.8$  Hz,  $\text{CH}_2$ ), 4.09 (1H, s, NH), 4.43 (1H, d,  $J = 12.4$  Hz,  $\text{CH}_2$ ), 6.62 (1H, td,  $J = 1.2, 8.0$  Hz, ArH), 6.74 (2H, td,  $J = 1.2, 8.4$  Hz, ArH), 6.81 (1H, d,  $J = 9.2$  Hz, ArH), 7.03 (1H, td,  $J = 1.6, 7.6$  Hz, ArH), 7.12 (1H, dd,  $J = 2.4, 8.8$  Hz, ArH), 7.43 (1H, d,  $J = 2.4$  Hz, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta$  28.60 (CH), 29.63, 32.07, 32.76 ( $\text{CH}_2$ ), 33.56 (C), 36.68, 40.04, 42.76, 45.49 ( $\text{CH}_2$ ), 46.03 ( $\text{CH}_2\text{CO}$ ), 49.60 ( $\text{CH}_2$ ), 112.23, 116.91, 118.51, 119.56, 125.25, 125.29 (ArCH), 127.91 (ArC), 128.52 (ArCH), 130.25, 138.25, 142.89, 151.45 (ArC), 169.60 (CO). HRMS: Calcd for  $\text{C}_{29}\text{H}_{34}\text{Cl}_2\text{N}_2\text{O}_2$  ( $\text{M}+\text{H}$ ) $^+$  513.2070, found ( $\text{M}+\text{H}$ ) $^+$  513.2069.

**1-(Furan-3-carbonyl)-piperidin-4-one, 119**,  $\text{C}_{10}\text{H}_{11}\text{NO}_3$ , MW 193.20,



To a solution of 4-piperidone hydrochloride monohydrate (320 mg, 2.1 mmol) in DCM (7 mL), was added MP-Carbonate resin (0.8 g). This was stirred at room temperature for 2 h. In a separate flask, 3-furoic acid (280 mg, 2.52 mmol), *N,N*-4-dimethylaminopyridine (cat.), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.2 g, 6.3 mmol) and TEA (0.25 mL) were dissolved in DCM (7 mL) and stirred at room temperature for 2 h. The two reaction mixtures were then combined and stirred at room temperature for a further 18 h. The resulting solution was then filtered to remove the resin. HCl (1M, 20 mL) was then added to the filtrate, which was extracted with DCM (3 x 30 mL). The organic layers were washed with, 20 mL),  $\text{NaHCO}_3$  (20 mL) then brine (20 mL). The organic layers were combined, dried ( $\text{MgSO}_4$ ), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to yield the title compound as a cream oil, 225 mg, 63 % yield. LCMS:  $t_r = 1.96$  min (50 % MeOH in water at 0.5 mL/min),  $m/z$   $\text{M}+\text{H}$  194.16, HPLC:  $t_r = 1.82$  min (90 % acetonitrile in water at 1.0 mL/min), >99 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  2.41 (4H, t,  $J = 6.2$  Hz,  $\text{CH}_2$ ), 3.85 (4H, t,  $J = 6.2$  Hz,  $\text{CH}_2$ ), 6.50 (1H, dd,  $J = 1.0, 2.0$  Hz, ArH), 7.37 (1H, t,  $J = 1.7$  Hz, ArH), 7.68 (1H, dd,  $J = 1.0, 1.7$  Hz, ArH).

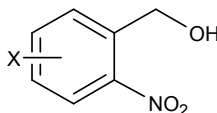
**(4-[2-(2,4-Dichloro-phenoxy)-phenylamino]-piperidin-1-yl)-furan-3-yl-methanone, 120**, C<sub>22</sub>H<sub>20</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>, MW 431.31,



A solution of 2-(2,4-dichloro-phenoxy)-phenylamine (165 mg, 0.7 mmol), 1-(furan-3-carbonyl)-piperidin-4-one (125 mg, 0.65 mmol), sodium triacetoxyborohydride (206 mg, 0.97 mmol) and AcOH (0.1 mmol, 3.3 mmol) in DCE (5 mL) was stirred at r.t. for 6 days. NaHCO<sub>3</sub> (20 mL) was then added and the mixture was extracted with EtOAc (3 x 20 mL). The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % ethyl acetate in hexane) to afford a brown oil, 62 mg, 22 % yield. LCMS:  $t_r$  = 6.3 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min),  $m/z$  M+H 431.41, HPLC:  $t_r$  = 3.35 min (90 % acetonitrile in water at 0.8 mL/min), 99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz, 50 °C):  $\delta$  1.38-1.48 (2H, m, CH<sub>2</sub>), 2.09 (2H, dd,  $J$  = 3.6, 12.8 Hz, CH<sub>2</sub>), 3.19 (2H, t,  $J$  = 11.6 Hz, CH<sub>2</sub>), 3.56-3.63 (1H, m, CH), 4.10 (1H, s, NH), 4.20 (1H, s, CH<sub>2</sub>), 6.52 (1H, d,  $J$  = 2.0 Hz, CH=CH-O-CH), 6.64 (1H, td,  $J$  = 1.2, 7.2 Hz, ArH), 6.76 (2H, m, ArH), 6.82 (1H, d,  $J$  = .4 Hz, ArH), 7.04 (1H, td,  $J$  = 1.6, 7.6 Hz, ArH), 7.13 (1H, dd,  $J$  = 2.8, 8.8 Hz, ArH), 7.40 (1H, t,  $J$  = 1.6 Hz, CH=CH-O-CH), 7.45 (1H, d,  $J$  = 2.4 Hz, ArH), 7.67 (1H, t,  $J$  = 0.8 Hz, C=CH-O). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz, 50 °C):  $\delta$  29.69, 32.56, 44.00 (CH<sub>2</sub>), 49.82 (CH), 110.02, 112.51, 117.20, 118.73, 119.56 (ArH), 121.19 (ArC), 125.37, 125.46, 127.98 (ArCH), 128.71 (ArC), 130.41, 138.38 (ArCH), 142.89, 143.14, 143.30, 151.30 (ArC), 163.76 (CO).

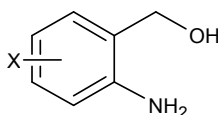
### Experimental Details: Chapter 8: Synthesis of Benzylamine Linked 17 $\beta$ -HSD3 Inhibitors

#### General Procedure for the Reduction of Substituted 2-Nitrobenzaldehyde



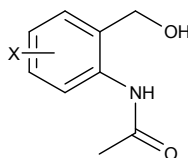
A solution of the desired substituted 2-nitrobenzaldehyde in EtOH (5 mL/mmol) was cooled to 0°C and to this was added NaBH<sub>4</sub> (1.5 eq) and the resulting solution was stirred at r.t. for 2 h. The EtOH was removed *in vacuo* and sat. NH<sub>4</sub>Cl solution was added and the mixture was then extracted with DCM and dried (MgSO<sub>4</sub>). It was then evaporated *in vacuo* to yield the desired substituted 2-nitrobenzylalcohol.

### General Procedure for the Reduction of the Substituted 2-Nitrobenzylalcohol



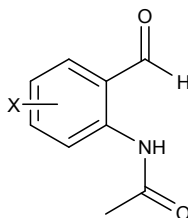
This procedure is as described by Matsuo *et al.*<sup>65</sup> To a refluxing mixture of iron (5.5 eq.) and ammonium chloride (0.7 eq.) in a 10:1 mixture of EtOH: H<sub>2</sub>O, the substituted 2-nitrobenzylalcohol (1 eq.) was added. This reaction mixture was stirred at reflux for between 1 and 4 h, and followed by TLC, it was then allowed to cool to room temperature and the solvent was removed *in vacuo*. The residue was re-dissolved in DCM and washed with sat. aqueous sodium bicarbonate. The organic layer was dried (MgSO<sub>4</sub>) filtered and evaporated *in vacuo* to afford the desired 2-aminobenzylalcohol.

### General Procedure for the Acylation of Substituted 2-Aminobenzylalcohols



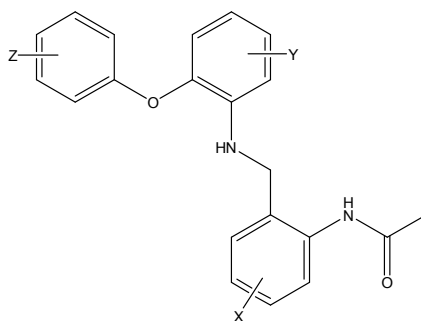
To a solution of the substituted 2-aminobenzylalcohol and TEA (3 eq) in DCM (10 mL/1mmol) at 0 °C, was added acetyl chloride (6 eq.). The resulting solution was allowed to warm to r.t. and stirred for 18 h. NaHCO<sub>3</sub> was then added and the crude mixture was repeatedly extracted with DCM (with a trace of MeOH). The organic layers were then washed with 1M HCl. The organic layers were combined and dried (MgSO<sub>4</sub>) filtered and evaporated *in vacuo*. The resulting solid was redissolved in MeOH (40 mL/1mmol) and to this was added NaOH (3 eq) and the reaction was stirred at r.t. for 2 h. The solvent was evaporated *in vacuo* and H<sub>2</sub>O was added, the crude mixture was repeatedly extracted with EtOAc. The organic layers were combined and dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo* to obtain the desired 2-acetamide benzylalcohol.

### General Procedure for the Dess-Martin Periodinane Oxidation of Alcohols



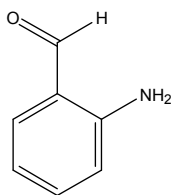
This procedure is based upon work by Dess *et al.*<sup>92, 93</sup> To a solution of the 2-acetamide alcohol in DCM (50 mL/1mmol) was added Dess-Martin Periodinane (1.5 eq.) the resulting solution was stirred at r.t for 10 min. Sodium thiosulphate (4.5 eq) in NaHCO<sub>3</sub> was then added and the mixture was repeatedly extracted with DCM. The organic layers were combined, dried (MgSO<sub>4</sub>) filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography to afford the desired aldehyde.

**General procedure for the Reductive Amination of the Substituted Diphenylether Aniline with the Substituted 2-acetamide benzaldehyde**



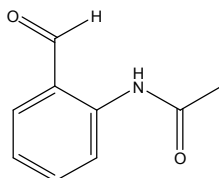
To a solution of the diphenylether aniline (1.5 eq) and aldehyde (1 eq) in DCE (2 mL/1mmol) was added acetic acid (3 eq) and sodium triacetoxyborohydride (2.5 eq). The resulting reaction mixture was stirred at r.t. for 2-18 h.  $\text{NaHCO}_3$  was then added and repeatedly extracted with DCM. The organic layers were combined, dried ( $\text{MgSO}_4$ ) filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography to afford the desired compound.

**2-Amino-benzaldehyde, 124**,  $\text{C}_7\text{H}_7\text{NO}$ , MW 121.14,



Using the general procedure for the reduction of the 2-nitrobenzylalcohol the desired compound was isolated as a yellow oil, 794 mg, 99 % yield. R.f. 0.35 (DCM), LCMS:  $t_r$  = 3.04 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min),  $m/z$   $\text{M}^+\text{H}$  122.15,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  6.12 (2H, s,  $\text{NH}_2$ ), 6.63 (1H, d,  $J$  = 8.4 Hz, ArH), 6.70-6.76 (1H, m, ArH), 7.26-7.32 (1H, m, ArH), 7.46 (1H, dd,  $J$  = 1.5, 7.7 Hz, ArH), 9.85 (1H, s, CHO). This compound has been previously synthesised using a similar method by Malecki *et al.*<sup>150</sup>

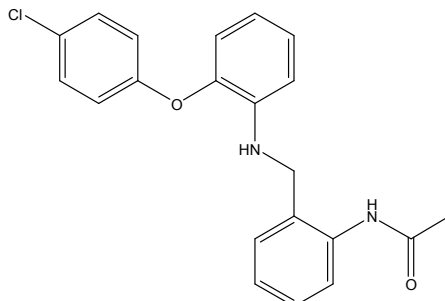
***N*-(2-Formyl-phenyl)-acetamide, 125**,  $\text{C}_9\text{H}_9\text{NO}_2$ , MW 163.17,



Using the general procedure for the acylation of substituted 2-aminobenzylalcohols the title compound was obtained as a yellow solid, 190 mg, 71% yield. R.f. 0.68 (DCM), m.p. 54-57 °C, LCMS:  $t_r$  = 1.67 min (90 % MeOH in water),  $m/z$   $\text{M}+\text{H}$  164.1,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  2.24 (3H, s,  $\text{CH}_3$ ), 7.21 (1H, td,  $J$  = 1.2, 7.7 Hz, ArH), 7.56-7.63 (1H, m, ArH), 7.65 (1H, dd,  $J$  = 1.5, 7.7 Hz, ArH), 8.72 (1H, d,  $J$  = 8.4 Hz, ArH), 9.85

(1H, s, CHO), 11.12 (1H, s, NH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz):  $\delta$  25.6 ( $\text{CH}_3$ ), 119.9 (ArCH), 121.5 (ArC), 123.0, 136.2, 136.4 (ArCH), 141.0 (ArC), 169.8 (CO), 195.7 (CHO). HRMS: Calcd for  $\text{C}_9\text{H}_9\text{NO}_2$  ( $\text{M}+\text{H}$ ) $^+$  164.0706, found ( $\text{M}+\text{H}$ ) $^+$  164.0699.

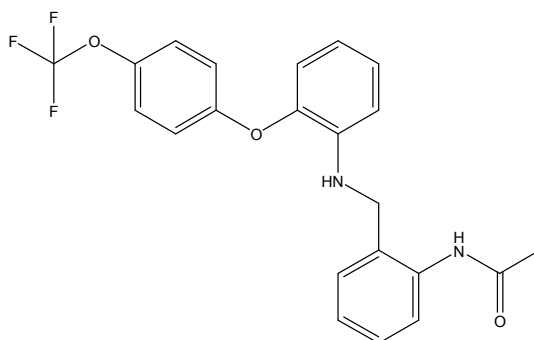
***N*-(2-([2-(4-Chloro-phenoxy)-phenylamino]-methyl)-phenyl)-acetamide, 126,**  
 $\text{C}_{21}\text{H}_{19}\text{ClN}_2\text{O}_2$ , MW 366.84,



To a solution of 2-(4-chloro-phenoxy)-phenylamine (0.128 g, 0.58 mmol) and *N*-(2-formyl-phenyl)-acetamide (0.19 g, 1.16 mmol) in DCE (2.6 mL) was added acetic acid (0.25 mL) and sodium triacetoxyborohydride (0.31 g, 1.45 mmol). The resulting reaction mixture was heated in a CEM microwave for 10 minutes at 140  $^{\circ}\text{C}$ .<sup>85</sup>  $\text{NaHCO}_3$  was then added and the mixture was repeatedly extracted with EtOAc. The organic layers were combined, dried ( $\text{MgSO}_4$ ) filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100% DCM in hexane) to afford the title compound as a white solid, 80 mg, 38 % yield. R.f. 0.33 (DCM), m.p. 194-196  $^{\circ}\text{C}$ , LCMS:  $t_r$  = 1.36 min (95 % MeOH in  $\text{H}_2\text{O}$ ),  $m/z$  M-H 365.4, HPLC:  $t_r$  = 5.1 min (90 % acetonitrile in  $\text{H}_2\text{O}$ , 0.5mL/min), 98 %,  $^1\text{H}$  NMR (DMSO, 400 MHz):  $\delta$  2.02 (3H, s,  $\text{CH}_3$ ), 4.27 (2H, d,  $J$  = 5.6 Hz,  $\text{CH}_2$ ), 5.95 (1H, s, NH), 6.46 (1H, d,  $J$  = 8.4 Hz, ArH), 6.55 (1H, td,  $J$  = 0.8, 7.6 Hz, ArH), 6.84 (1H, dd,  $J$  = 1.6, 8.0, ArH), 6.89-6.95 (3H, m, ArH), 7.07-7.11 (1H, m, ArH), 7.16-7.22 (2H, m, ArH), 7.35-7.41 (3H, m, ArH), 9.47 (1H, br.s, NHCO).  $^{13}\text{C}$  NMR (DMSO, 101 MHz): 23.2 ( $\text{CH}_3$ ), 42.6 ( $\text{CH}_2$ ), 111.7, 116.0, 118.3, 120.2, 125.2, 125.3, 125.6 (ArCH), 126.0 (ArC), 126.7, 126.9, 129.6 (ArCH), 133.5, 135.8, 140.5, 141.6, 156.7 (ArC), 168.5 (CO). HRMS: Calcd for  $\text{C}_{21}\text{H}_{19}\text{ClN}_2\text{O}_2$  ( $\text{M}+\text{H}$ ) $^+$  367.1208, found ( $\text{M}+\text{H}$ ) $^+$  367.1204. Anal. calcd for  $\text{C}_{21}\text{H}_{19}\text{ClN}_2\text{O}_2$ : C 68.76, H 5.22, N 7.64 %. Found: C 69.0, H 5.28, N 7.52%.

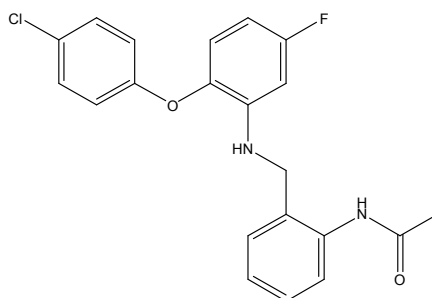


***N*-(2-([2-(4-Trifluoromethoxy-phenoxy)-phenylamino]-methyl)-phenyl)-acetamide, 127**, C<sub>22</sub>H<sub>19</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub>, MW 416.39,



Using the general procedure for the reductive amination of the substituted diphenylether aniline with the substituted 2-acetamide benzaldehyde the desired compound was isolated as a white solid, 155 mg, 55 % yield. R.f. 0.4 (1:1, EtOAc: Hexane), m.p. 98-100 °C, LCMS:  $t_r$  = 1.07 min (95 % MeOH in H<sub>2</sub>O),  $m/z$  M-H 415.09, HPLC:  $t_r$  = 2.22 min (90 % acetonitrile in H<sub>2</sub>O), 99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.94 (3H, s, CH<sub>3</sub>), 4.3 (3H, s, CH<sub>2</sub> and NH), 6.79-6.96 (5H, m, ArH), 7.06-7.17 (3H, m, ArH), 7.25-7.34 (3H, m, ArH), 8.01 (1H, d,  $J$  = 8.15, ArH), 8.54 (1H, br.s, NHCO). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): δ 24.5 (CH<sub>3</sub>), 47.8 (CH<sub>2</sub>), 113.8, 118.1, 119.6, 119.7 (ArCH), 122.5 (ArC), 122.8, 124.6, 125.7 (ArCH), 127.6 (ArC), 128.9, 129.6 (ArCH), 137.5, 139.9, 143.9, 144.4 (ArC), 155.9 (OCF<sub>3</sub>), 168.5 (CO). <sup>19</sup>F NMR (CDCl<sub>3</sub>, 376 MHz): δ -58.29 (OCF<sub>3</sub>). HRMS: Calcd for C<sub>22</sub>H<sub>19</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub> (M+Na)<sup>+</sup> 439.1240, found (M+Na)<sup>+</sup> 439.1240. Anal. calcd for C<sub>22</sub>H<sub>19</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub>: C 63.46, H 4.60 N 6.73 %. Found: C 63.5, H 4.62, N 7.0 %.

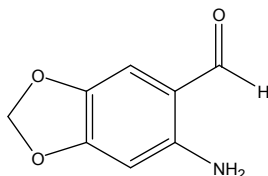
***N*-(2-([2-(4-Chloro-phenoxy)-5'-fluoro-phenylamino]-methyl)-phenyl)-acetamide, 128**, C<sub>21</sub>H<sub>18</sub>ClF<sub>2</sub>N<sub>2</sub>O<sub>2</sub>, MW 384.83,



Using the general procedure for the reductive amination of the substituted diphenylether aniline with the substituted 2-acetamide benzaldehyde the desired compound was isolated as a white solid, 68 mg, 27 % yield. R.f. 0.4 (1:1, EtOAc: Hexane), m.p. 178-180 °C, LCMS:  $t_r$  = 0.98 min (95 % MeOH in H<sub>2</sub>O),  $m/z$  M-H 383.28, HPLC:  $t_r$  = 2.87 min (90 % acetonitrile in H<sub>2</sub>O), 99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 2.03 (3H, s, CH<sub>3</sub>), 4.23 (2H, d,  $J$  = 5.0Hz, CH<sub>2</sub>), 4.42 (1H, t,  $J$  = 4.9 Hz, NH), 6.41-6.58 (2H, m, ArH), 6.80-6.88 (3H, m, ArH), 7.10 (1H, t,  $J$  = 7.2 Hz, ArH), 7.22-7.32 (4H, m, ArH), 7.86 (1H, d,  $J$  = 7.9 Hz, ArH), 8.19 (1H, br.s, NHCO). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): δ 24.3

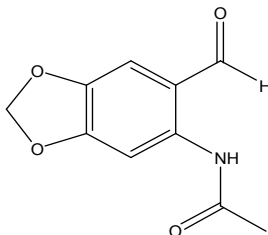
(CH<sub>3</sub>), 46.8 (CH<sub>2</sub>), 100.7 (d,  $J$  = 28.1Hz, ArH), 104.6 (d,  $J$  = 23.7Hz, ArCH), 118.0 (ArCH), 120.8 (d,  $J$  = 10.0Hz, ArCH), 123.6 (ArCH), 128.1 (d,  $J$  = 11.8Hz, ArC), 128.9, 129.4, 129.9 (ArCH), 136.9, 139.2 (ArC), 141.3 (d,  $J$  = 10.6Hz, ArC), 156.3, 158.8, 162.3 (ArC), 168.7 (CO). <sup>19</sup>F NMR (CDCl<sub>3</sub>, 376 MHz):  $\delta$  -115.43-115.57 (m, ArF). HRMS: Calcd for C<sub>21</sub>H<sub>18</sub>ClF<sub>2</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup> 383.0968, found (M+H)<sup>+</sup> 383.0965.

**6-Amino-benzo[1,3]dioxole-5-carbaldehyde, 129**, C<sub>8</sub>H<sub>7</sub>NO<sub>3</sub>, MW 165.15,



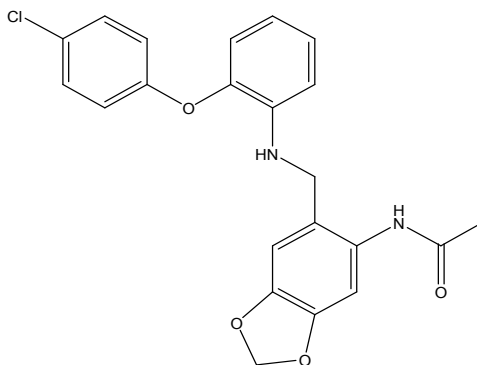
Using the general procedure for the reduction of substituted 2-nitrobenzylalcohol the desired compound was obtained as a brown solid, 360 mg, 85 % yield. R.f. 0.67 (DCM), <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  5.90 (2H, s, CH<sub>2</sub>), 6.11 (1H, s, ArH), 6.29 (2H, br.s, NH), 6.79 (1H, s, ArH), 9.57 (1H, s, CHO).

**N-(6-Formyl-benzo[1,3]dioxol-5-yl)-acetamide, 130**, C<sub>10</sub>H<sub>9</sub>NO<sub>4</sub>, MW 207.18,



Using the general procedure for the acylation of substituted 2-aminobenzylalcohols the title compound was obtained as a dark yellow solid, 180 mg, 78 % yield. m.p. 133-137 °C, R.f. 0.35 (DCM), m.p. 133-137 °C, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  2.21 (3H, s, CH<sub>3</sub>), 6.05 (2H, s, CH<sub>2</sub>), 6.98 (1H, s, ArH), 8.34 (1H, s, ArH), 9.65 (1H, s, CHO), 11.46 (1H, s, NH).

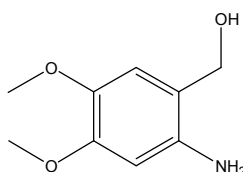
**N-(6-[2-(4-Chloro-phenoxy)-phenylamino]-methyl-benzo[1,3]dioxol-5-yl)-acetamide, 131**, C<sub>22</sub>H<sub>19</sub>ClN<sub>2</sub>O<sub>4</sub>, MW 410.85,



Using the general procedure for the reductive amination of the substituted diphenylether aniline with the substituted 2-acetamide benzaldehyde the desired compound was

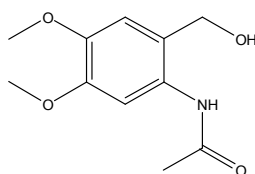
isolated as a light cream solid, 540 mg, 29 % yield. R.f. 0.75 (DCM), m.p. 154-156 °C, LCMS:  $t_r$  = 1.3 min (95 % MeOH in water),  $m/z$  MH 409.45, HPLC:  $t_r$  = 4.7 min (90 % acetonitrile in H<sub>2</sub>O), 98 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  1.95 (3H, s, CH<sub>3</sub>), 4.18 (2H, s, NHCH<sub>2</sub>), 4.23 (1H, s, NH), 5.93 (2H, s, CH<sub>2</sub>O), 6.74 (1H, s, ArH), 6.77-6.81 (1H m, ArH), 6.86-6.89 (3H, m, ArH), 7.10 (1H, td,  $J$  = 4.0, 8.8 Hz, ArH), 7.24-7.27 (2H, m, ArH), 7.44 (1H, s, ArH), 8.23 (1H, s, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta$  24.2 (CH<sub>3</sub>), 47.2 (CH<sub>2</sub>NH), 101.4 (CH<sub>2</sub>O), 105.3, 109.1, 113.4, 118.5, 119.2, 119.4 (ArCH), 121.7 (ArC), 125.4 (ArCH), 128.0 (ArC), 129.8 (ArCH), 131.1, 139.6, 143.7, 144.7, 147.4, 155.9 (ArC), 168.3 (CO). HRMS: Calcd for C<sub>22</sub>H<sub>19</sub>ClN<sub>2</sub>O<sub>4</sub> (M+H)<sup>+</sup> 409.0961, found (M+H)<sup>+</sup> 409.0957.

**(2-Amino-4,5-dimethoxy-phenyl)-methanol, 135a**, C<sub>9</sub>H<sub>13</sub>NO<sub>3</sub>, MW 183.20,



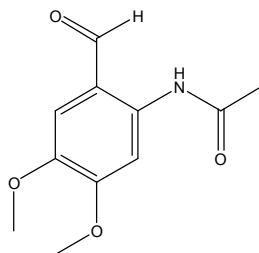
Using the general procedure for the reduction of the substituted 2-nitrobenzylalcohol the desired compound was obtained as a brown oil, 764 mg, 93 % yield. R.f. 0.17 (EtOAc), <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  3.30 (2H, br.s, NH<sub>2</sub>), 3.78 (3H, s, OCH<sub>3</sub>), 3.81 (3H, s, OCH<sub>3</sub>), 4.58 (2H, s, CH<sub>2</sub>), 6.29 (1H, s, ArH), 6.63 (1H, s, ArH).

**N-(2-Hydroxymethyl-4,5-dimethoxy-phenyl)-acetamide, 136a**, C<sub>11</sub>H<sub>15</sub>NO<sub>4</sub>, MW 225.24,



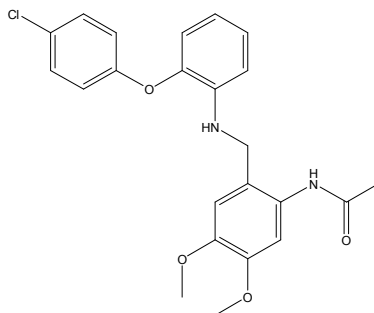
Using the general procedure for the acylation of substituted 2-aminobenzylalcohols the title compound was obtained as a yellow, waxy solid, 277 mg, 59 % yield. R.f. 0.48 (EtOAc), <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  2.14 (3H, s, CH<sub>3</sub>), 2.85, (1H, br.s, OH), 3.82 (3H, s, OCH<sub>3</sub>), 3.84 (3H, s, OCH<sub>3</sub>), 4.56 (2H, s, CH<sub>2</sub>), 6.69 (1H, s, ArH), 7.46 (1H, s, ArH).

***N*-(2-Formyl-4,5-dimethoxy-phenyl)-acetamide, 137a**, C<sub>11</sub>H<sub>13</sub>NO<sub>4</sub>, MW 223.23,



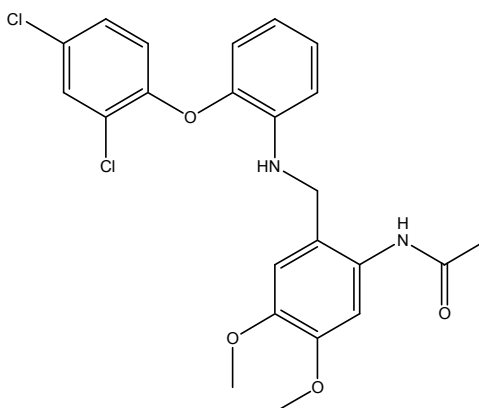
Using the general procedure for the Dess-Martin Periodinane oxidation of alcohols the title compound was obtained as a pale yellow solid, 137 mg, 50 % yield. R.f. 0.22 (EtOAc), m.p. 175-178 °C, HPLC  $t_r$  = 1.05min (90 % acetonitrile in H<sub>2</sub>O) 96 %, LCMS  $t_r$  = 0.86min (95 % MeOH in H<sub>2</sub>O) M-H 221.77, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 2.23 (3H, s, CH<sub>3</sub>), 3.90 (3H, s, OCH<sub>3</sub>), 3.98 (3H, s, OCH<sub>3</sub>), 7.02 (1H, s, ArH), 8.46 (1H, s, ArH), 9.74 (1H, s, CHO), 11.32 (1H, br.s, NH).

***N*-(2-[2-(4-Chloro-phenoxy)-phenylamino]-methyl-4,5-dimethoxy-phenyl)-acetamide, 136**, C<sub>23</sub>H<sub>23</sub>ClN<sub>2</sub>O<sub>4</sub>, MW 426.89,



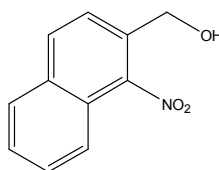
Using the general procedure for the reductive amination of the substituted diphenylether aniline with the substituted 2-acetamide benzaldehyde the resulting reaction mixture was stirred at r.t. for 2 h, then it was subjected to microwave heating for 5 min at 140 °C. NaHCO<sub>3</sub> was then added and the mixture was repeatedly extracted with DCM. The organic layers were combined, dried (MgSO<sub>4</sub>) filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (100 % EtOAc) to afford the title compound as a cream solid, 20 mg, 11 % yield. R.f. 0.44 (EtOAc), m.p. 117-118 °C, LCMS:  $t_r$  = 0.97 min (95 % MeOH in water),  $m/z$  M-H 425.16, HPLC:  $t_r$  = 1.99 min (90 % acetonitrile in H<sub>2</sub>O), 98 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.94 (3H, s, CH<sub>3</sub>), 3.81 (3H, s, OCH<sub>3</sub>), 3.85 (3H, s, OCH<sub>3</sub>), 4.22 (3H, br.s, CH<sub>2</sub> and NH), 6.74-6.90 (6H, m, ArH), 7.06-7.12 (1H, m, ArH), 7.21-7.26 (2H, m, ArH), 7.57 (1H, s, ArH), 8.26 (1H, br.s, NHCO). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): δ 24.4 (CH<sub>3</sub>), 47.2 (CH<sub>2</sub>), 56.11, 56.27 (OCH<sub>3</sub>), 107.4, 112.5, 113.6, 118.5, 119.3, 119.6 (ArCH), 120.2 (ArC), 125.6 (ArCH), 128.1 (ArC), 129.9, (ArCH), 130.6, 139.9, 143.7, 148.7, 156.0 (ArC), 168.5 (CO). HRMS: Calcd for C<sub>23</sub>H<sub>23</sub>ClN<sub>2</sub>O<sub>4</sub> (M+Na)<sup>+</sup> 449.1230, found (M+Na)<sup>+</sup> 449.1239.

**N-(2-([2-(2,4-Dichloro-phenoxy)-phenylamino]-methyl)-4,5-dimethoxy-phenyl)-acetamide, 139**, C<sub>23</sub>H<sub>22</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>4</sub>, MW 461.34,



2-(2, 4-Dichloro-phenoxy)-phenylamine hydrochloride (135 mg, 0.47 mmol) was dissolved in DCM (10 mL) and to this was added K<sub>2</sub>CO<sub>3</sub> (128 mg, 0.94 mmol) the reaction was then stirred at r.t. for 30 min. H<sub>2</sub>O was added to the reaction and the mixture was extracted with DCM. The organic layers were combined, dried (MgSO<sub>4</sub>) filtered and evaporated *in vacuo*. The resulting free amine was dissolved in DCE (2 mL) and to this was added N-(2-formyl-4,5-dimethoxy-phenyl)-acetamide (69 mg, 0.31 mmol) acetic acid (0.12 mL) and sodium triacetoxymethylborohydride (164 mg, 0.8 mmol). The resulting reaction mixture was stirred at r.t. for 2 h. NaHCO<sub>3</sub> was then added, and repeatedly extracted with DCM. The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-50 % EtOAc in hexane) to afford the title compound as an off-white solid, 55 mg, 38 % yield. R.f. 0.58 (EtOAc), m.p. 128-131 °C, LCMS: *t*<sub>r</sub> = 1.1 min (95 % MeOH in water), *m/z* M-H 459.24, HPLC: *t*<sub>r</sub> = 2.2 min (90 % acetonitrile in H<sub>2</sub>O), 96 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.98 (3H, s, CH<sub>3</sub>), 3.83 (3H, s, OCH<sub>3</sub>), 3.87 (3H, s, OCH<sub>3</sub>), 4.26 (2H, s, CH<sub>2</sub>), 4.34 (1H, br.s, NH), 6.75-6.77 (3H, m, ArH), 6.83-6.91 (2H, m, ArH), 7.02-7.11 (1H, m, ArH), 7.15 (1H, dd, *J* = 1.7, 8.7Hz, ArH), 7.61 (1H, s, ArH), 8.31 (1H, br.s, NHCO). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 24.5 (CH<sub>3</sub>), 47.2 (CH<sub>2</sub>), 56.1, 56.3 (OCH<sub>3</sub>), 104.0 (ArC), 107.4, 112.5, 113.6, 118.0, 119.1, 120.0, 125.4 (ArCH), 125.5, 125.8 (ArC), 128.1 (ArCH), 129.0 (ArC), 130.6 (ArCH), 139.1, 143.7, 146.0, 148.7, 151.5 (ArC), 168.5 (CO). HRMS: Calcd for C<sub>23</sub>H<sub>22</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>4</sub> (M+H)<sup>+</sup> 461.1029, found (M+H)<sup>+</sup> 461.1028.

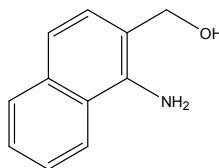
**(1-Nitro-naphthalen-2-yl)-methanol, 134b**, C<sub>11</sub>H<sub>9</sub>NO<sub>3</sub>, MW 203.19,



Using the general procedure for the reduction of substituted 2-nitrobenzaldehyde the desired product was obtained as a dark yellow solid, 1 g, >99 % yield. R.f. 0.59

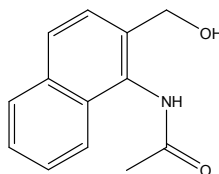
(EtOAc), m.p 78-80 °C,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  2.32 (1H, br.s, OH), 4.82 (2H, s,  $\text{CH}_2$ ), 7.48-7.65 (3H, m, ArH), 7.81-7.90 (2H, m, ArH), 7.99 (1H, d,  $J = 8.4$ , ArH).

**(1-Amino-naphthalen-2-yl)-methanol, 135b**,  $\text{C}_{11}\text{H}_{11}\text{NO}$ , MW 173.21,



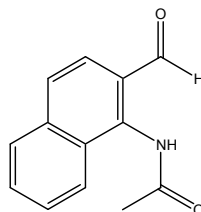
Using the general procedure for the reduction of the substituted 2-nitrobenzylalcohol, the desired product was obtained, 730 mg, 86 % yield. R.f. 0.62 (EtOAc), LCMS:  $t_r = 1.3$  min (80 % MeOH in water),  $m/z$  M-H 171.88, HPLC:  $t_r = 2.19$  min (70 % acetonitrile in water), 87 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  4.85 (2H, s,  $\text{CH}_2$ ), 7.21-7.25 (2H, m, ArCH), 7.41-7.47 (2H, m, ArH), 7.74-7.85 (2H, m, ArH).

**N-(2-Hydroxymethyl-naphthalen-1-yl)-acetamide, 136b**,  $\text{C}_{13}\text{H}_{13}\text{NO}_2$ , MW 215.25,



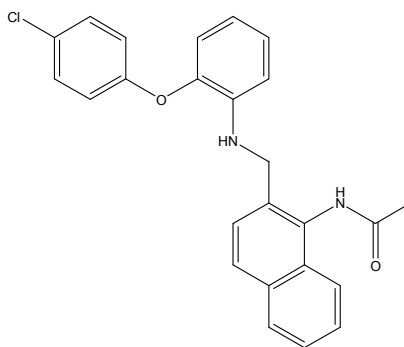
Using the general procedure for the acylation of substituted 2-aminobenzylalcohols the desired product was obtained as a yellow solid, 377 mg, 82 % yield. R.f. 0.58 (EtOAc), m.p. 105-108 °C, HPLC  $t_r = 1.45$  min (90 % acetonitrile in  $\text{H}_2\text{O}$ ) >99 %, LCMS  $t_r = 0.92$  min (95 % MeOH in  $\text{H}_2\text{O}$ ) M+Na 237.90,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  2.32 (3H, s,  $\text{CH}_3$ ), 3.29 (1H, br.s, OH), 4.63 (2H, s,  $\text{CH}_2$ ), 7.40-7.44 (3H, m, ArH), 7.77-7.80 (3H, m, ArH).

**N-(2-Formyl-naphthalen-1-yl)-acetamide, 137b**,  $\text{C}_{13}\text{H}_{11}\text{NO}_2$ , MW 213.23,



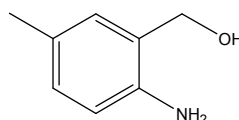
Using the general procedure for the Dess-Martin Periodinane oxidation of alcohols the desired product was obtained, 59 mg, 77 % yield. R.f. 0.45 (EtOAc),  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  2.28 (3H, s,  $\text{CH}_3$ ), 7.55-7.92 (5H, m, ArH), 7.99-8.03 (1H, m, ArH), 9.31 (1H, br.s, NH), 10.18 (1H, s, CHO).

***N*-(2-[2-(4-Chloro-phenoxy)-phenylamino]-methyl-naphthalen-1-yl)-acetamide, 140**, C<sub>25</sub>H<sub>21</sub>ClN<sub>2</sub>O<sub>2</sub>, MW 416.90,



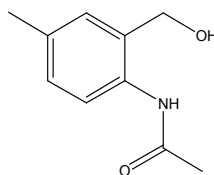
Using the general procedure for the reductive amination of the substituted diphenylether aniline with the substituted 2-acetamide benzaldehyde the desired compound was isolated, 28 mg, 24 % yield. R.f. 0.38 (EtOAc), m.p. 153-155 °C, LCMS:  $t_r$  = 1.14 min (95 % MeOH in water),  $m/z$  M-H 415.34, HPLC:  $t_r$  = 2.26 min (90 % acetonitrile in water), 96 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 2.28 (3H, s, CH<sub>3</sub>), 4.42 (2H, s, CH<sub>2</sub>), 4.53 (1H, br.s, NH), 6.63-6.73 (2H, m, ArH), 6.83-6.91 (3H, m, ArH), 6.97-7.03 (1H, m, ArH), 7.21-7.24 (2H, m, ArH), 7.42-7.51 (4H, m, ArH), 7.75 (1H, d,  $J$  = 8.4Hz, ArH), 7.80-7.85 (2H, m, ArH and NHCO). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): 23.5 (CH<sub>3</sub>), 45.4 (CH<sub>2</sub>), 112.4, 117.7, 118.5, 119.6, 122.6, 125.5, 126.0, 126.1 (ArCH), 127.8 (ArC), 128.2, 128.4, 129.8 (ArCH), 130.5, 130.6, 133.4, 133.7, 140.2, 142.9, 156.5 (ArC), 169.4 (CO). HRMS: Calcd for C<sub>25</sub>H<sub>21</sub>ClN<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup> 439.1184, found (M+H)<sup>+</sup> 439.1190.

**(2-Amino-5-methyl-phenyl)-methanol, 135c**, C<sub>8</sub>H<sub>11</sub>NO, MW 137.18,



Using the general procedure for the reduction of the substituted 2-nitrobenzylalcohol the desired compound was obtained as a brown solid, 162 mg, 79 % yield. R.f. 0.45 (EtOAc), m.p. 118-121 °C, LCMS:  $t_r$  = 0.93 min (95 % MeOH in water),  $m/z$  M+H 137.80, HPLC:  $t_r$  = 1.53 min (90 % acetonitrile in H<sub>2</sub>O), 96 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 2.22 (3H, s, CH<sub>3</sub>), 4.02 (2H, br.s, NH), 4.64 (2H, s, CH<sub>2</sub>), 6.62 (1H, d,  $J$  = 9.3 Hz, ArH), 6.89-6.95 (2H, m, ArH), 7.25 (1H, s, OH).

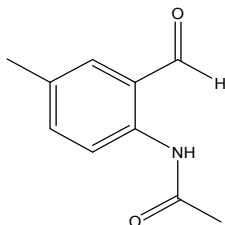
***N*-(2-Hydroxymethyl-4-methyl-phenyl)-acetamide, 136c**, C<sub>10</sub>H<sub>13</sub>NO<sub>2</sub>, MW 179.22,



Using the general procedure for the acylation of substituted 2-aminobenzylalcohols the title compound was obtained as a cream solid, 180 mg, 79 % yield. R.f. 0.35 (EtOAc),

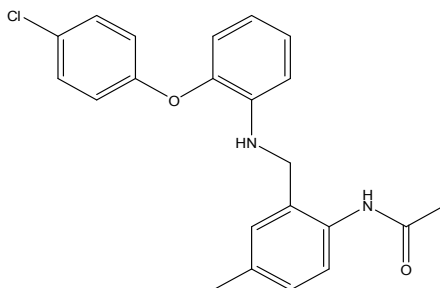
m.p. 134-136 °C (from hexane), LCMS  $t_r$  = 1.4 min (80 % MeOH in H<sub>2</sub>O) M+Na 210.99, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  2.17 (3H, s, CH<sub>3</sub>), 2.29 (3H, s, CH<sub>3</sub>), 4.63 (2H, d,  $J$  = 4.9Hz, CH<sub>2</sub>), 7.02 (1H, s, ArH), 7.12 (1H, d,  $J$  = 8.4 Hz, ArH), 7.80 (1H, d,  $J$  = 8.2 Hz, ArH), 8.28 (1H, s, NH).

***N*-(2-Formyl-4-methyl-phenyl)-acetamide, 137c**, C<sub>10</sub>H<sub>11</sub>NO<sub>2</sub>, MW 177.20,



Using the general procedure for the Dess-Martin Periodinane oxidation of alcohols the title compound was obtained as a red oil, 44 mg, 25 % yield. R.f. 0.8 (10 % MeOH in DCM), <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  2.21 (3H, s, CH<sub>3</sub>), 2.36 (3H, s, CH<sub>3</sub>), 7.37-7.41 (2H, m, ArH), 8.55-8.61 (1H, m, ArH), 9.84 (1H, s, CHO), 10.98 (1H, br.s, NH).

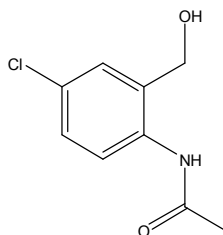
***N*-(2-[2-(4-Chloro-phenoxy)-phenylamino]-methyl-4-methyl-phenyl)-acetamide, 141**, C<sub>22</sub>H<sub>21</sub>ClN<sub>2</sub>O<sub>2</sub>, MW 380.87,



Using the general procedure for the reductive amination of the substituted diphenylether aniline with the substituted 2-acetamide benzaldehyde the desired compound was isolated as a light cream solid, 37 mg, 47 % yield. R.f. 0.35 (EtOAc), m.p. 138-140 °C (from hexane), LCMS:  $t_r$  = 1.21 min (95 % MeOH in water),  $m/z$  M+H 381.20, HPLC:  $t_r$  = 2.38 min (90 % acetonitrile in H<sub>2</sub>O), 96 %. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  1.97 (3H, s, CH<sub>3</sub>), 2.29 (3H, s, CH<sub>3</sub>), 4.25 (3H, s, CH<sub>2</sub> and NH), 6.76-6.93 (5H, m, ArCH), 7.07-7.13 (3H, m, ArH), 7.22-7.27 (2H, m, ArH), 7.83 (1H, d,  $J$  = 8.2Hz, ArH), 8.35 (1H, br.s, NH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz):  $\delta$  20.9, 24.5 (CH<sub>3</sub>), 47.5 (CH<sub>2</sub>), 113.6, 118.6, 119.3, 119.6, 123.1, 125.5 (ArCH), 128.0, 128.1 (ArC), 129.3, 129.9, 130.2 (ArCH), 134.4, 134.7, 139.9, 143.8, 156.0 (ArC), 168.5 (CO). HRMS: Calcd for C<sub>22</sub>H<sub>21</sub>ClN<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup> 381.1364, found (M+H)<sup>+</sup> 381.1365.

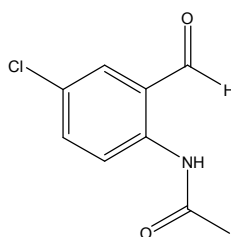


***N*-(4-Chloro-2-hydroxymethyl-phenyl)-acetamide, 136d**, C<sub>9</sub>H<sub>10</sub>ClNO<sub>2</sub>, MW 199.63,



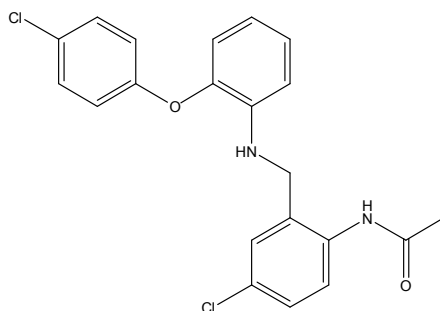
Using the general procedure for the acylation of substituted 2-aminobenzylalcohols the title compound was obtained as a cream wax, 1.12 g, 89 % yield. R.f. 0.35 (EtOAc), <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.46 (2H, s, NH and OH), 2.19 (3H, s, CH<sub>3</sub>), 4.60 (2H, s, CH<sub>2</sub>), 7.14-7.26 (2H, m, ArH), 7.94-7.98 (1H, m, ArH).

***N*-(4-Chloro-2-formyl-phenyl)-acetamide, 137d**, C<sub>9</sub>H<sub>8</sub>ClNO<sub>2</sub>, MW 197.62,



Using the general procedure for the Dess-Martin Periodinane oxidation of alcohols the title compound was obtained as a red solid, 383 mg, 70 % yield. R.f. 0.72 (10% MeOH in EtOAc), m.p. 152-154 °C, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 2.24 (3H, s, CH<sub>3</sub>), 7.55 (1H, dd, *J* = 2.5, 8.9 Hz, ArH), 7.61 (1H, dd, *J* = 2.5 Hz, ArH), 8.71 (1H, d, *J* = 9.2 Hz, ArH), 9.84 (1H, s, CHO), 11.00 (1H, br.s, NH).

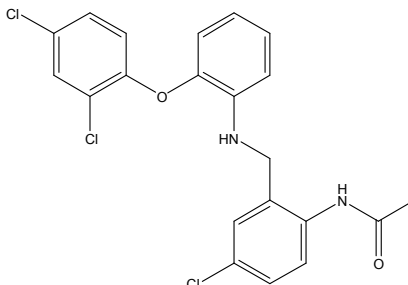
***N*-(4-chloro-2-[2-(4-chloro-phenoxy)-phenylamino]-methyl-phenyl)-acetamide, 142**, C<sub>21</sub>H<sub>18</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>, MW 401.29,



Using the general procedure for the reductive amination of the substituted diphenylether aniline with the substituted 2-acetamide benzaldehyde the desired compound was isolated as a light brown wax, 68mg, 26 % yield. R.f. 0.43 (1:1, EtOAc: Hexane), LCMS: *t*<sub>r</sub> = 1.21 min (95 % MeOH in water), *m/z* M-H 399.15, 401.1, HPLC: *t*<sub>r</sub> = 2.62 min (90 % acetonitrile in H<sub>2</sub>O), 97 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.95 (3H, s, CH<sub>3</sub>), 4.22 (3H, s, NH and CH<sub>2</sub>), 6.79-6.91 (5H, m, ArH), 7.07-7.13 (1H, m, ArH), 7.24-7.28 (4H, m, ArH), 7.98 (1H, d, *J* = 8.4 Hz, ArH), 8.56 (1H, br.s, NHCO). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 24.5 (CH<sub>3</sub>), 47.5 (CH<sub>2</sub>), 113.8, 118.7, 119.5, 119.9, 124.1,

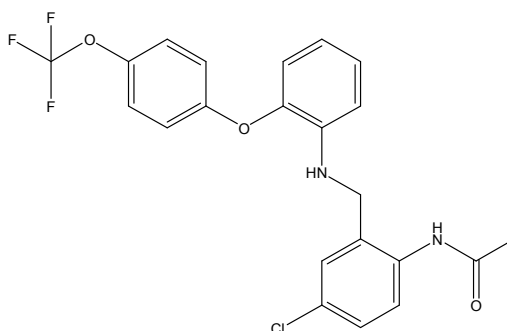
125.5, 128.7, 129.3 (ArCH), 129.5 (ArC), 129.9 (ArCH), 136.0, 139.4, 144.1, 155.9, 168.5, 200.5 (ArC), 205.1 (CO). HRMS: Calcd for  $C_{21}H_{18}Cl_2N_2O_2$  (M+H)<sup>+</sup> 401.0818, found (M+H)<sup>+</sup> 401.0803.

***N*-(4-Chloro-2-[2-(2,4-dichloro-phenoxy)-phenylamino]-methyl-phenyl)-acetamide, 143**,  $C_{21}H_{17}Cl_3N_2O_2$ , MW 435.73,



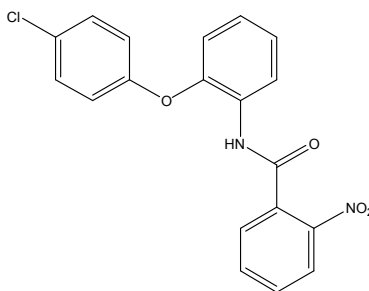
2-(2,4-Dichloro-phenoxy)-phenylamine hydrochloride (350 mg, 1.22 mmol) was dissolved in DCM (10 mL) and to this was added  $K_2CO_3$  (335 mg, 2.44 mmol), the reaction was then stirred at r.t. for 30 min.  $H_2O$  was then added and the mixture was extracted with DCM. The organic layers were combined, dried ( $MgSO_4$ ), filtered and evaporated *in vacuo*. The resulting amine was dissolved in DCE (2 mL) and to this was added *N*-(4-chloro-2-formyl-phenyl)-acetamide (160 mg, 0.81 mmol), acetic acid (0.15 mL) and sodium triacetoxyborohydride (0.43 g, 2.02 mmol). The resulting reaction mixture was stirred at r.t. for 2 h.  $NaHCO_3$  was then added and the mixture was repeatedly extracted with DCM. The organic layers were combined, dried ( $MgSO_4$ ), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-50% EtOAc in hexane) to afford the title compound as a cream solid, 210 mg, 60 % yield. R.f. 0.38 (EtOAc), m.p. 135-137 °C, LCMS:  $t_r$  = 1.3 min (95 % MeOH in water),  $m/z$  M-H 433.1, 435.1, HPLC:  $t_r$  = 2.8 min (90 % acetonitrile in  $H_2O$ ), 99 %,  $^1H$  NMR ( $CDCl_3$ , 270 MHz):  $\delta$  1.98 (3H, s,  $CH_3$ ), 4.28-4.29 (2H, m,  $CH_2$ ), 4.40 (1H, br.s, NH), 6.70-6.88 (4H, m, ArH), 7.04-7.10 (1H, m, ArH), 7.17 (1H, dd,  $J$  = 2.5 Hz, ArH), 7.25-7.27 (2H, m, ArH), 7.45 (1H, d,  $J$  = 2.5 Hz, ArH), 7.96-7.99 (1H, m, ArCH), 8.64 (1H, br.s. NH).  $^{13}C$  NMR ( $CDCl_3$ , 101 MHz):  $\delta$  24.6 ( $CH_3$ ), 47.4 ( $CH_2$ ), 113.8, 117.8, 119.7, 120.3, 124.1, 125.3 (ArCH), 125.9 (ArC), 128.2, 128.7, 129.3 (ArCH), 129.4, 129.6 (ArC), 130.7 (ArCH), 136.0, 138.6, 144.4, 151.1 (ArC), 168.5 (CO). HRMS: Calcd for  $C_{21}H_{17}Cl_3N_2O_2$  (M+H)<sup>+</sup> 435.0428, found (M+H)<sup>+</sup> 437.0387.

***N*-(4-Chloro-2-[2-(4-trifluoromethoxy-phenoxy)-phenylamino]-methyl-phenyl)-acetamide, 144**, C<sub>22</sub>H<sub>18</sub>ClF<sub>3</sub>N<sub>2</sub>O<sub>3</sub>, MW 450.84



Using the general procedure for the reductive amination of the substituted diphenylether aniline with the substituted 2-acetamide benzaldehyde the desired compound was isolated as a brown solid, 86 mg, 30 % yield. R.f. 0.35 (1:1, EtOAc: Hexane), m.p. 121-123 °C, LCMS:  $t_r$  = 1.07 min (95 % MeOH in water),  $m/z$  M-H 449.29, HPLC:  $t_r$  = 3.28 min (90 % acetonitrile in H<sub>2</sub>O), >99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.94 (3H, s, CH<sub>3</sub>), 4.23 (3H, s, CH<sub>2</sub> and NH), 6.80-6.96 (5H, m, ArH), 7.08-7.18 (3H, m, ArH), 7.25-7.30 (3H, m, ArH), 7.99 (1H, dd,  $J$  = 8.4 Hz, ArH), 8.54 (1H, br.s, NHCO). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): 24.4 (CH<sub>3</sub>), 47.4 (CH<sub>2</sub>), 113.8, 118.1, 119.6, 119.8, 112.8, 123.9, 125.5, 128.6, 129.2 (ArCH), 135.9, 139.3 (ArC), 155.6 (OCF<sub>3</sub>), 207.9 (CO). <sup>19</sup>F NMR (CDCl<sub>3</sub>, 376 MHz): δ -58.3 (OCF<sub>3</sub>). Anal. Calcd for C<sub>22</sub>H<sub>18</sub>ClF<sub>3</sub>N<sub>2</sub>O<sub>3</sub>: C 58.61, H 4.02, N 6.21 %. Found: C 58.1, H 4.12, N 5.96 %. HRMS: Calcd for C<sub>22</sub>H<sub>18</sub>ClF<sub>3</sub>N<sub>2</sub>O<sub>3</sub> (M+H)<sup>+</sup> 451.1031, found (M+H)<sup>+</sup> 451.1024.

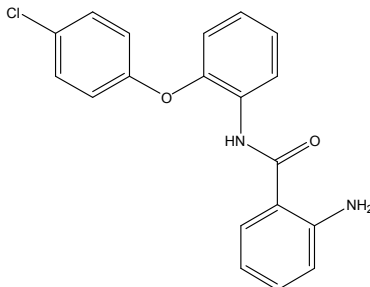
***N*-[2-(4-chloro-phenoxy)-phenyl]-2-nitro-benzamide, 145**, C<sub>19</sub>H<sub>13</sub>ClN<sub>2</sub>O<sub>4</sub>, MW 368.77,



To a solution of 2-(4-chloro-phenoxy)-phenylamine (200 mg, 0.91 mmol) in DCM (5 mL) at 0 °C was added 2-nitrobenzoyl chloride (338 mg, 1.82 mmol) and TEA (0.15 mL). The reaction was then stirred at r.t. for 30 min. NaHCO<sub>3</sub> was added and the mixture was extracted with DCM, dried (MgSO<sub>4</sub>) and purified by flash chromatography (0-100 % DCM in hexane) to yield the desired product as an off white oil, 258 mg, 77 % yield. LCMS:  $t_r$  = 1.91 min (80 % MeOH in water),  $m/z$  M-H 367.09, HPLC:  $t_r$  = 2.16 min (90 % acetonitrile in water), 90 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 6.83 (1H, dd,  $J$  = 1.4, 8.0 Hz, ArH), 6.91-6.99 (2H, m, ArH), 7.07 (1H, td,  $J$  = 1.6, 8.0 Hz, ArH), 7.17 (1H, t,  $J$  = 7.2Hz, ArH), 7.24-7.31 (2H, m, ArH), 7.48 (1H, dd,  $J$  = 1.4, 7.2Hz,

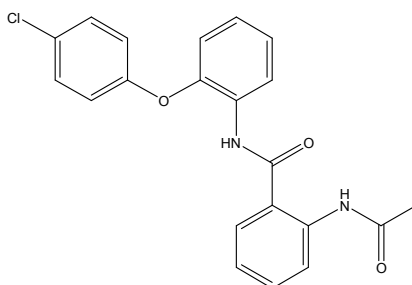
ArH), 7.58 (1H, td,  $J = 1.4, 7.5$  Hz, ArH), 7.65 (1H, dd,  $J = 1.1, 7.4$  Hz, ArH), 8.00-8.06 (2H, m, ArH and NH), 8.47 (1H, dd,  $J = 1.1, 8.0$  Hz, ArH).

**2-Amino-*N*-[2-(4-chloro-phenoxy)-phenyl]-benzamide, 146,** C<sub>19</sub>H<sub>15</sub>ClN<sub>2</sub>O<sub>2</sub>, MW 338.79,



Using the general procedure for the reduction of the substituted 2-nitrobenzylalcohol the desired product was obtained as a white wax, 143 mg, 62 % yield. R.f. 0.39 (DCM), LCMS:  $t_r = 2.67$  min (80 % MeOH in water),  $m/z$  M-H 337.05, HPLC:  $t_r = 2.59$  min (90 % acetonitrile in water), 98 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  5.55 (2H, br.s., NH<sub>2</sub>), 6.62-6.70 (2H, m, ArH), 6.87 (1H, dd,  $J = 1.4, 8.0$  Hz, ArH), 6.96-6.99 (2H, m, ArH), 7.04 (1H, td,  $J = 1.4, 7.5$  Hz, ArH), 7.15-7.25 (2H, m, ArH), 7.39-7.34 (3H, m, ArH), 8.34 (1H, br.s, NH), 8.50 (1H, dd,  $J = 1.4, 8.0$  Hz, ArH).

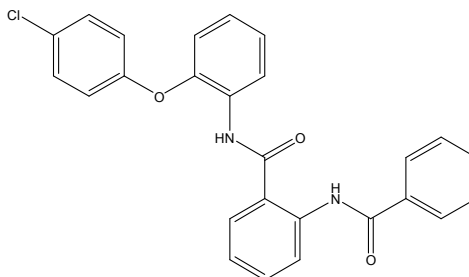
**2-Acetylamino-*N*-[2-(4-chloro-phenoxy)-phenyl]-benzamide, 147,** C<sub>21</sub>H<sub>17</sub>ClN<sub>2</sub>O<sub>3</sub>, MW 380.82,



2-Amino-*N*-[2-(4-chloro-phenoxy)-phenyl]-benzamide (120 mg, 0.36 mmol) was dissolved in DCM (3 mL) and cooled to 0 °C, to this was added acetyl chloride (0.05 mL, 0.72 mmol) and TEA (0.07 mL, 1.1 mmol). The resulting solution was stirred at r.t. for 1 h, NaHCO<sub>3</sub> was then added and the mixture extracted repeatedly with DCM. The organic portions were then washed with HCl (1M) and dried (MgSO<sub>4</sub>). The crude mixture was then purified by flash chromatography (0-50 % EtOAc in DCM) to yield the desired product as a white solid, 65 mg, 48 % yield. R.f. 0.46 (25 % EtOAc in DCM), m.p. 158-160 °C, LCMS:  $t_r = 1.05$  min (95 % MeOH in water),  $m/z$  M-H 379.00, HPLC:  $t_r = 2.48$  min (90 % acetonitrile in water), 99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  2.2 (3H, s, CH<sub>3</sub>), 6.87 (1H, dd,  $J = 1.6, 8.3$  Hz, ArH), 6.96-7.01 (2H, m, ArH), 7.05-7.13 (2H, m, ArH), 7.19 (1H, td,  $J = 1.6, 7.7$  Hz, ArH), 7.29-7.34 (2H, m, ArH), 7.45-7.52 (2H, m, ArH), 8.40-8.44 (2H, m, ArH and NH), 8.58 (1H, d,  $J = 15.7$  Hz,

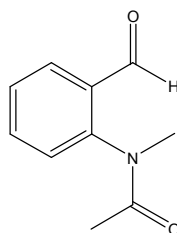
ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz): 28.0 ( $\text{CH}_3$ ), 117.7, 120.3 (ArCH), 121.0 (ArC), 121.5, 121.9, 123.1, 124.4, 125.2, 126.6 (ArCH), 128.9, 129.6 (ArC), 130.2, 133.2 (ArCH), 139.9, 146.4, 154.6 (ArC), 167.3, 169.1 (CO). HRMS: Calcd for  $\text{C}_{21}\text{H}_{17}\text{ClN}_2\text{O}_3$  ( $\text{M}+\text{H}$ ) $^+$  381.1000, found ( $\text{M}+\text{H}$ ) $^+$  381.0994.

**2-Benzoylamino-*N*-[2-(4-chloro-phenoxy)-phenyl]-benzamide, 148**,  $\text{C}_{26}\text{H}_{19}\text{ClN}_2\text{O}_3$ , MW 442.89,



2-Amino-*N*-[2-(4-chloro-phenoxy)-phenyl]-benzamide (120 mg, 0.36 mmol) was dissolved in DCM (3 mL) and cooled to  $0^\circ\text{C}$ , to this was added benzoyl chloride (0.08 mL, 0.72 mmol) and TEA (0.07 mL, 1.1 mmol). The resulting solution was stirred at r.t. for 1 h and  $\text{NaHCO}_3$  was then added and the mixture was repeatedly extracted with DCM. This was then washed with HCl (1M) and dried ( $\text{MgSO}_4$ ). The crude mixture was then purified by flash chromatography (0-100 % DCM in hexane) to yield the desired product as a white solid, 60 mg, 38 % yield. R.f. 0.46 (DCM), m.p.  $183\text{--}185^\circ\text{C}$ , LCMS:  $t_r = 1.29$  min (95 % MeOH in water),  $m/z$  M-H 440.97, HPLC:  $t_r = 3.89$  min (90 % acetonitrile in water), >99 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  6.88 (1H, d,  $J = 7.6$  Hz, ArH), 6.99 (2H, d,  $J = 2.0$  Hz, ArH), 7.08-7.15 (2H, m, ArH), 7.21 (1H, t,  $J = 7.2$  Hz, ArH), 7.31 (2H, dd,  $J = 0.8, 6.8$  Hz, ArH), 7.50-7.59 (5H, m, ArH and NH), 8.04 (2H, dd,  $J = 1.2, 4.8$  Hz, ArH), 8.49-8.51 (2H, m, ArH), 8.83 (1H, d,  $J = 8.0$  Hz, ArH), 11.89 (1H, br.s, NH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz):  $\delta$  117.6, 120.2 (ArCH), 128.8 (ArC), 121.6, 121.9, 123.1, 124.3, 125.1, 127.4, 128.8 (ArCH), 128.9, 129.4 (ArC), 130.1, 131.9, 133.3 (ArCH), 134.7, 140.3, 146.3, 154.6 (ArC), 165.6, 167.3 (CO). HRMS: Calcd for  $\text{C}_{26}\text{H}_{19}\text{ClN}_2\text{O}_3$  ( $\text{M}+\text{H}$ ) $^+$  443.1157, found ( $\text{M}+\text{H}$ ) $^+$  443.1162.

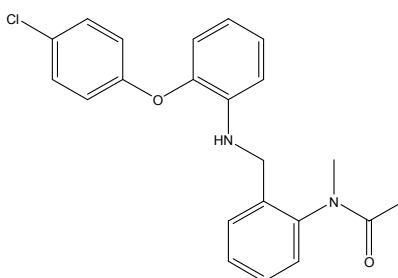
***N*-(2-Formyl-phenyl)-*N*-methyl-acetamide, 149**,  $\text{C}_{10}\text{H}_{11}\text{NO}_2$ , MW 177.20,<sup>151</sup>



To a solution of *N*-(2-formyl-phenyl)-acetamide (100 mg, 0.61 mmol) in DMF (10 mL) was added NaH (60 % dispersion in mineral oil, 30 mg, 0.73 mmol). After 1h had elapsed MeI (0.08 mL, 1.2 mmol) was added and this was then stirred at r.t. under  $\text{N}_2$

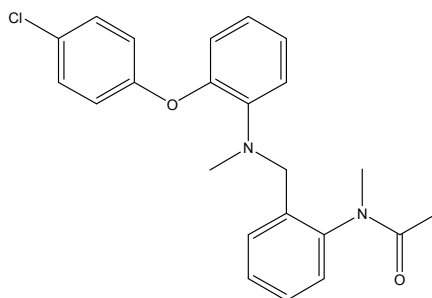
for 3 days. This was poured onto water (20 mL), extracted with EtOAc and dried (MgSO<sub>4</sub>). The crude product was purified by flash chromatography (0-100 % DCM in hexane) to yield the desired product as an off white wax, 60 mg, 56 % yield. R.f. 0.35 (DCM), LCMS:  $t_r$  = 1.0 min (95 % MeOH in water),  $m/z$  M+H 177.80, HPLC:  $t_r$  = 1.0 min (95 % MeOH in water), 97 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  1.79 (3H, s, CH<sub>3</sub>CO), 3.28 (3H, s, CH<sub>3</sub>N), 7.26-7.29 (1H, m, ArH), 7.50-7.55 (1H, m, ArH), 7.69 (1H, td,  $J$  = 1.6, 7.5 Hz, ArH), 7.97 (1H,  $J$  = 1.6, 7.7 Hz, ArH), 10.13 (1H, s, CHO).

***N*-(2-([2-(4-Chloro-phenoxy)-phenylamino]-methyl)-phenyl)-*N*-methyl-acetamide, 150**, C<sub>22</sub>H<sub>21</sub>ClN<sub>2</sub>O<sub>2</sub>, MW 380.87,



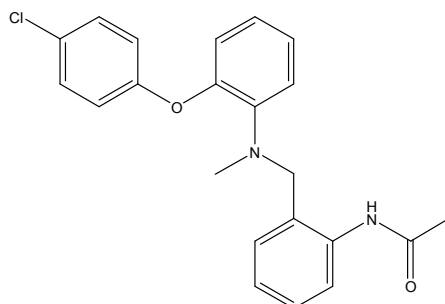
To a solution of 2-(4-chloro-phenoxy)-phenylamine (174 mg, 0.78 mmol) and *N*-(2-formyl-phenyl)-*N*-methyl-acetamide (70 mg, 0.39 mmol) in DCE (2mL) was added NaHB(OAc)<sub>3</sub> (210 mg, 0.98 mmol) and AcOH (0.07 mL). The resulting solution was stirred at r.t. for 2 h. NaHCO<sub>3</sub> was then added and the mixture was extracted with DCM and dried (MgSO<sub>4</sub>). The crude product was purified by flash chromatography (0-100 % DCM in hexane) to yield the desired product as a brown oil, 65 mg, 44 % yield. R.f. 0.51 (DCM), LCMS:  $t_r$  = 1.19 min (95 % MeOH in water),  $m/z$  M-H 378.93, HPLC:  $t_r$  = 2.64 min (90 % acetonitrile in water), 98 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  1.78 (3H, s, CH<sub>3</sub>CO), 3.24 (3H, s, CH<sub>3</sub>N), 4.25-4.29 (2H, m, CH<sub>2</sub>), 6.59 (1H, dd,  $J$  = 1.4, 8.0 Hz, ArH), 6.67 (1H, td,  $J$  = 1.4, 7.7 Hz, ArH), 6.83 (1H, dd,  $J$  = 1.4, 7.7 Hz, ArH), 6.88-6.92 (2H, m, ArH), 7.00 (1H, td,  $J$  = 1.6, 7.7 Hz, ArH), 7.11-7.17 (1H, m, ArH), 7.23-7.26 (2H, m, ArH), 7.31-7.34 (2H, m, ArH), 7.41-7.44 (1H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): 26.5, 36.6 (CH<sub>3</sub>), 43.7 (CH<sub>2</sub>), 117.7, 118.7, 119.5, 125.4 (ArCH), 127.9 (ArC), 128.5, 128.9, 129.1, 129.7 (ArCH), 136.3, 139.9, 142.5, 142.8, 156.1 (ArC), 170.8 (CO). HRMS: Calcd for C<sub>22</sub>H<sub>21</sub>ClN<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup> 381.1364, found (M+H)<sup>+</sup> 381.1378.

***N*-[2-([(2-(4-Chloro-phenoxy)-phenyl]-methyl-amino)-methyl)-phenyl]-*N*-methyl-acetamide, **151**, C<sub>23</sub>H<sub>23</sub>ClN<sub>2</sub>O<sub>2</sub>, MW 394.89,**



*N*-(2-([2-(4-chloro-phenoxy)-phenylamino]-methyl)-phenyl)-acetamide, (100 mg, 0.27 mmol) in DMF (10 mL) was cooled to 0 °C, to this was added NaH (35mg, 0.81mmol) and the resulting solution was stirred for 1 h. MeI (0.05 mL, 0.81 mmol) was then added and the solution stirred for a further 18 h. The reaction mixture was then poured onto water, extracted with EtOAc and dried (MgSO<sub>4</sub>). NMR analysis showed the crude product to be a mixture of product and the related mono-methylated compound. Preparative-HPLC was used for purification to yield the desired product as a white wax, 25 mg, 23 % yield. Please note *N*-(2-([2-(4-chloro-phenoxy)-phenylamino]-methyl)-phenyl)-*N*-methyl-acetamide (**157**) was also isolated, 46 mg, 43 % yield. R.f. 0.45 (EtOAc), LCMS: *t*<sub>r</sub> = 5.6 min (80 % MeOH in water), *m/z* M+H 395.18, HPLC: *t*<sub>r</sub> = 3.75 min (90 % acetonitrile in water), 98 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.70 (3H, s, CH<sub>3</sub>), 2.66 (3H, s, NCH<sub>3</sub>), 3.09 (3H, s, NCH<sub>3</sub>), 4.10 (2H, s, CH<sub>2</sub>), 6.67-6.73 (2H, m, ArH), 6.96 (2H, d, *J* = 3.9 Hz, ArH), 7.04-7.27 (8H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): δ 22.0, 36.3, 39.7 (CH<sub>3</sub>), 54.9 (CH<sub>2</sub>), 117.8, 119.7, 122.2, 122.5, 125.6 (ArCH), 127.1 (ArC), 142.4, 145.0, 147.4 (ArC), 170.8 (CO). HRMS: Calcd for C<sub>23</sub>H<sub>23</sub>ClN<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup> 395.1521, found (M+H)<sup>+</sup> 395.1533.

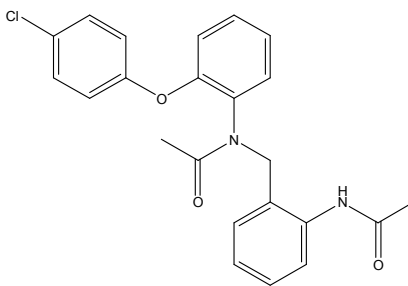
***N*-[2-([(2-(4-Chloro-phenoxy)-phenyl]-methyl-amino)-methyl)-phenyl]-acetamide, **152**, C<sub>22</sub>H<sub>21</sub>ClN<sub>2</sub>O<sub>2</sub>, MW 380.87,**



To a solution of *N*-(2-[2-(4-chloro-phenoxy)-phenylamino]-methyl-phenyl)-acetamide, (100 mg, 0.27 mmol), paraformaldehyde (81 mg, 2.7 mmol) and NaBH<sub>4</sub> (55 mg, 1.35 mmol) in THF (5 mL) was added TFA (1.3 mL). The resulting solution was stirred at r.t. for 18 h.<sup>94</sup> This was then poured into NaOH solution (25 %) with ice chips, extracted with DCM and dried (MgSO<sub>4</sub>).<sup>94</sup> The crude product was then purified by

flash chromatography (0-100 % EtOAc in hexane) to produce the desired compound as a colourless oil, 64 mg, 62 % yield. R.f. 0.66 (EtOAc), LCMS:  $t_r$  = 4.02 min (80 % MeOH in water),  $m/z$  M+H 379.12, HPLC:  $t_r$  = 2.88 min (90 % MeOH in water), 99 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.99 (3H, s,  $\text{CH}_3$ ), 2.65 (3H, s,  $\text{CH}_3\text{N}$ ), 4.19 (2H, s,  $\text{CH}_2$ ), 6.81 (1H, dd,  $J$  = 1.6, 8.4 Hz, ArH), 6.93-6.95 (2H, m, ArH), 6.99-7.04 (2H, m, ArH), 7.10 (1H, td,  $J$  = 1.6, 8.0 Hz, ArH), 7.14-7.15 (1H, m, ArH), 7.23-7.32 (4H, m, ArH), 8.27 (1H, d,  $J$  = 8.4 Hz, ArH), 10.12 (1H, br.s, NHCO).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz): 24.8, 40.7 ( $\text{CH}_3$ ), 59.5 ( $\text{CH}_2$ ), 118.7, 120.3, 120.9, 121.3, 123.2, 124.1, 124.6 (ArCH), 125.1 (ArC), 128.6 (ArCH), 129.0 (ArC), 130.1 (ArCH), 138.6, 142.4, 150.8, 155.1 (ArC), 168.6 (CO). HRMS: Calcd for  $\text{C}_{22}\text{H}_{21}\text{ClN}_2\text{O}_2$  (M+H) $^+$  381.1364, found (M+H) $^+$  381.1363.

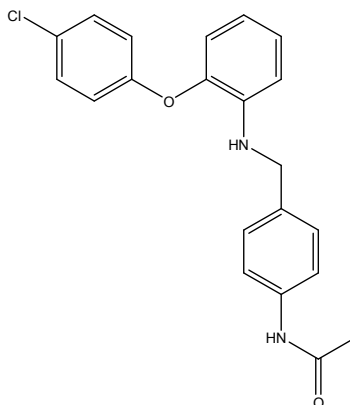
***N*-(2-Acetylamino-benzyl)-*N*-[2-(4-chloro-phenoxy)-phenyl]-acetamide, 153,**  
 $\text{C}_{23}\text{H}_{21}\text{ClN}_2\text{O}_3$ , MW 408.88,



A solution of *N*-(2-[2-(4-chloro-phenoxy)-phenylamino]-methyl-phenyl)-acetamide (100 mg, 0.27 mmol) in DCM (5 mL) and cooled to 0 °C, to this was added TEA (0.2 mL) and acetyl chloride (0.34 mL, 0.81 mmol) the resulting solution was stirred at r.t. for 1 h. Saturated  $\text{NaHCO}_3$  was added, extracted with DCM and dried ( $\text{MgSO}_4$ ). The crude product was purified by flash chromatography (0-100 % EtOAc in hexane) and preparative HPLC to yield the desired product as an off white waxy solid, 53 mg, 48 % yield. R.f. 0.54 (EtOAc), LCMS:  $t_r$  = 2.17 min (95 % MeOH in water),  $m/z$  M-H 407.15, HPLC:  $t_r$  = 2.40 min (90 % acetonitrile in water), 95 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.93 (3H, s,  $\text{CH}_3$ ), 2.24 (3H, s,  $\text{CH}_3$ ), 4.80 (2H, s,  $\text{CH}_2$ ), 7.02-7.12 (2H, m, ArH), 7.21-7.30 (4H, m, ArH), 8.20 (1H, dd,  $J$  = 7.7 Hz, ArH), 9.89 (1H, s, NHCO).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz): 22.1, 24.6 ( $\text{CH}_3$ ), 49.8 ( $\text{CH}_2$ ), 118.1, 120.7, 122.1, 123.0, 124.0 (ArCH), 125.0 (ArC), 129.2 (ArCH), 129.8 (ArC), 130.1, 130.2, 131.5 (ArCH), 137.7, 153.2, 153.8 (ArC), 169.5, 172.7 (CO). HRMS: Calcd for  $\text{C}_{23}\text{H}_{21}\text{ClN}_2\text{O}_3$  (M+Na) $^+$  431.1313, found (M+Na) $^+$  431.1105.

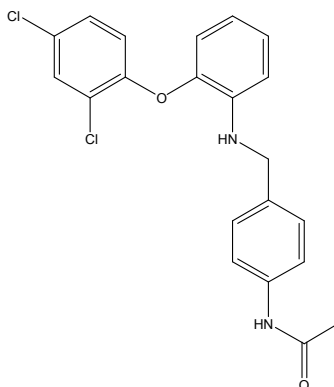


***N*-(4-([2-(4-chloro-phenoxy)-phenylamino]-methyl)-phenyl)-acetamide, 154,**  
 $C_{21}H_{19}ClN_2O_2$ , MW 366.84,



Using the general procedure for the reductive amination of the substituted diphenylether aniline with the substituted 2-acetamide benzaldehyde the desired compound was isolated as a light pink solid, 195 mg, 78 % yield. R.f. 0.55 (5 % MeOH in DCM), m.p. 137-139 °C, LCMS:  $t_r$  = 1.39 min (95 % MeOH in  $H_2O$ ),  $m/z$  M-H 365.48, HPLC:  $t_r$  = 2.0 min (90 % acetonitrile in  $H_2O$ ), 98 %,  $^1H$  NMR ( $CDCl_3$ , 270 MHz):  $\delta$  2.15 (3H, s,  $CH_3$ ), 4.30 (2H, d,  $J$  = 5.7Hz,  $CH_2$ ), 6.60-6.69 (2H, m, ArH), 6.82 (1H, dd,  $J$  = 1.5, 7.7Hz, ArH), 6.86-6.91 (2H, m, ArH), 6.96-7.02 (1H, m, ArH), 7.12 (1H, s, NH), 7.21-7.26 (4H, m, ArH), 7.41-7.44 (2H, m, ArH).  $^{13}C$  NMR ( $CDCl_3$ , 101 MHz):  $\delta$  24.6 ( $CH_3$ ), 47.2 ( $CH_2$ ), 111.9, 117.1, 118.5, 119.3, 120.1, 125.3 (ArCH), 127.6 (ArC), 127.9, 129.6 (ArCH), 135.1, 136.8, 140.1, 142.6, 156.2 (ArC), 168.2 (CO). HRMS: Calcd for  $C_{21}H_{19}ClN_2O_2$  ( $M+Na$ ) $^+$  389.1025, found ( $M+Na$ ) $^+$  389.1028. Anal. calcd for  $C_{21}H_{19}ClN_2O_2$ : C 68.76, H 5.22 N 7.64 %. Found: C 68.5, H 5.26, N 7.61 %.

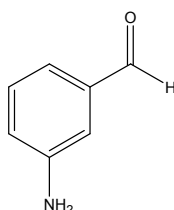
***N*-(4-([2-(2,4-Dichloro-phenoxy)-phenylamino]-methyl)-phenyl)-acetamide, 155,**  
 $C_{21}H_{18}Cl_2N_2O_2$ , MW 401.29,



To a solution of 2-(2,4-dichloro-phenoxy)-phenylamine hydrochloride (0.15g, 0.52mmol) in DCM (10mL) was added  $K_2CO_3$  (0.22g, 1.04mmol), the resulting solution was stirred at r.t for 30 min. Water was then added and the mixture was extracted with DCM. The organic layers were combined, dried ( $MgSO_4$ ), filtered and evaporated *in vacuo*. The resulting amine was dissolved in DCE (3mL) and to this was added 4-acetamidobenzaldehyde (0.126 g, 0.0.75mmol), acetic acid (0.11 mL) and

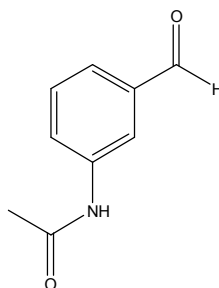
sodium triacetoxyborohydride (0.27g, 1.3mmol). The resulting reaction mixture was stirred at r.t. for 2h. NaHCO<sub>3</sub> was then added and the mixture was repeatedly extracted with DCM. The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % EtOAc in hexane) to afford the title compound as a white wax, 142 mg, 69 % yield. R.f. 5.8 (EtOAc), LCMS:  $t_r$  = 1.2 min (95 % MeOH in H<sub>2</sub>O),  $m/z$  M-H 399.03, 401.04, HPLC:  $t_r$  = 2.42 min (90 % acetonitrile in H<sub>2</sub>O), 98 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  2.15 (3H, s, CH<sub>3</sub>), 4.31 (2H, s, CH<sub>2</sub>), 4.59 (1H, br.s, NH), 6.59-6.68 (2H, m, ArH), 6.75 (1H, dd,  $J$  = 1.5, 7.9Hz, ArH), 6.80 (1H, d,  $J$  = 8.7 Hz, ArH), 6.96-7.02 (1H, m, ArH), 7.12 (1H, dd,  $J$  = 2.5, 8.9 Hz, ArH), 7.22-7.31 (1H, m, ArH), 7.41-7.45 (2H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz):  $\delta$  24.7 (CH<sub>3</sub>), 47.3 (CH<sub>2</sub>), 112.2, 117.1, 118.5, 119.4, 120.2 (ArCH), 125.3 (ArC), 125.5, 127.9, 128.0 (ArCH), 128.2 (ArC), 130.4 (ArCH), 135.1, 136.9, 139.7, 142.7, 151.8 (ArC), 168.4 (CO). HRMS: Calcd for C<sub>21</sub>H<sub>18</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup> 399.0673, found (M+H)<sup>+</sup> 399.0674. Anal. calcd for C<sub>21</sub>H<sub>18</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub> C 62.85, H 4.52 N 6.98 %. Found: C 62.7, H 4.52, N 6.92 %.

**3-Amino-benzaldehyde, 156**, C<sub>7</sub>H<sub>7</sub>NO, MW 121.14,



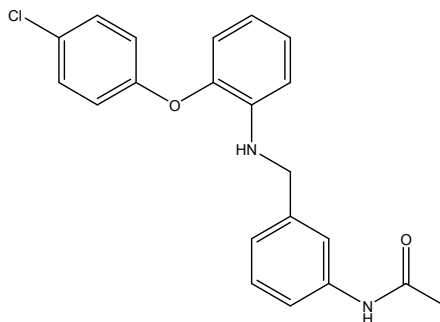
Using the general procedure for the reduction of the substituted 2-nitrobenzylalcohol the desired compound was obtained as a yellow solid, 1.7 g, 71 % yield. R.f. 0.25 (DCM). Due to the instability of this compound it was not possible to carry out further purification or analysis and so the product was used crude in the following reactions.

**N-(3-Formyl-phenyl)-acetamide, 157**, C<sub>9</sub>H<sub>9</sub>NO<sub>2</sub>, MW 163.17



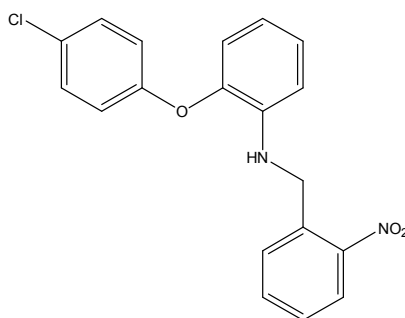
Using the general procedure for the acylation of substituted 2-aminobenzylalcohols the title compound was obtained as a cream oil, 250 mg, 37 % yield. R.f. 0.43 (10 % MeOH in DCM), <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  2.19 (3H, s, CH<sub>3</sub>), 7.42 (1H, t,  $J$  = 7.9 Hz, ArH), 7.56 (1H, d,  $J$  = 7.7 Hz, ArH), 7.83-7.86 (1H, m, ArH), 8.03 (1H, s, ArH), 8.60 (1H, s, NH), 9.90 (1H, s, CHO).

***N*-(3-[2-(4-Chloro-phenoxy)-phenylamino]-methyl-phenyl)-acetamide, 158,**  
C<sub>21</sub>H<sub>19</sub>ClN<sub>2</sub>O<sub>2</sub>, MW 366.84,



Using the general procedure for the reductive amination of the substituted diphenylether aniline with the substituted 2-acetamide benzaldehyde the desired compound was isolated as a cream solid, 110 mg, 63 % yield. R.f. 0.6 (EtOAc), m.p. 187-190 °C, LCMS:  $t_r$  = 1.39 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min),  $m/z$  M-H 365.55, HPLC:  $t_r$  = 1.89 min (90 % acetonitrile in H<sub>2</sub>O), 93 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 2.13 (3H, s, CH<sub>3</sub>), 4.32 (2H, d,  $J$  = 5.4 Hz, CH<sub>2</sub>), 4.55 (1H, d,  $J$  = 5.4 Hz, NH), 6.61-6.66 (2H, m, ArH), 6.81-6.84 (1H, m, ArH), 6.68-6.92 (2H, m, ArH), 6.98-7.04 (2H, m, ArH), 7.21-7.27 (4H, m, ArH and NH), 7.38-7.43 (2H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): δ 24.6 (CH<sub>3</sub>), 47.5 (CH<sub>2</sub>), 112.1, 117.2, 118.4, 118.7, 119.4, 123.0, 125.4 (ArCH), 127.7 (ArC), 129.0, 129.7, 129.9 (ArC), 138.2, 140.1, 140.4, 142.7, 156.3 (ArC), 168.5 (CO). HRMS: Calcd for C<sub>21</sub>H<sub>19</sub>ClN<sub>2</sub>O<sub>2</sub> (M+Na)<sup>+</sup> 389.1027, found (M+Na)<sup>+</sup> 389.1021.

**[2-(4-Chloro-phenoxy)-phenyl]-2-nitro-benzylamine, 159,** C<sub>19</sub>H<sub>15</sub>ClN<sub>2</sub>O<sub>3</sub>, MW 354.79,

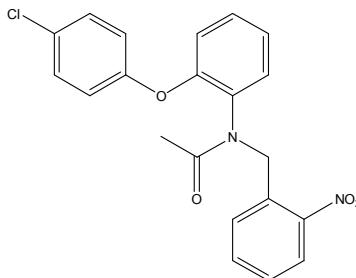


To a solution of 2-(4-chloro-phenoxy)-phenylamine (150 mg, 0.68 mmol) and 2-nitrobenzaldehyde (310 mg, 2.04 mmol) in DCE (3.5 mL) was added acetic acid (0.36 mL) and sodium triacetoxyborohydride (0.36 g, 1.7 mmol). The resulting reaction mixture was heated in a microwave at 140 °C for 10 min.<sup>85</sup> NaHCO<sub>3</sub> was then added and the mixture was repeatedly extracted with EtOAc. The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-100 % EtOAc in hexane) to afford the title compound as a yellow wax, 194 mg, 77 % yield. R.f. 0.63 (1:1, EtOAc: Hexane), LCMS:  $t_r$  = 1.66 min (95 % MeOH in H<sub>2</sub>O),  $m/z$  M+H 355.48, HPLC:  $t_r$  = 6.6 min (90

% acetonitrile in H<sub>2</sub>O), 92 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 4.75 (2H, s, CH<sub>2</sub>), 4.97 (1H, s, NH), 6.52 (1H, dd, *J* = 1.2, 7.9 Hz, ArH), 6.66 (1H, td, *J* = 1.5, 7.7 Hz, ArH), 6.83-6.99 (4H, m, ArH), 7.21-7.27 (2H, m, ArH), 7.37-7.44 (1H, m, ArH), 7.54-7.57 (2H, m, ArH), 8.05 (1H, dd, *J* = 1.0, 7.7 Hz, ArH).

***N*-[2-(4-Chloro-phenoxy)-phenyl]-*N*-(2-nitro-benzyl)-acetamide, 160,**

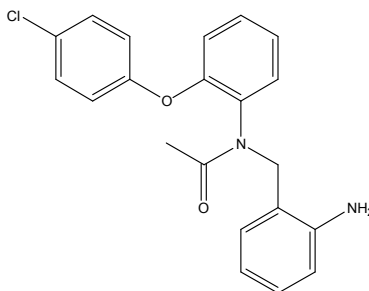
C<sub>21</sub>H<sub>17</sub>ClN<sub>2</sub>O<sub>4</sub>, MW 396.82,



Using the general procedure for the acylation of substituted 2-aminobenzylalcohols the title compound was obtained as a brown oil, 92 mg, 48 % yield. R.f. 0.21 (1:1, DCM:hexane), LCMS: *t*<sub>r</sub> = 5.12 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min), *m/z* M+H 397.48, HPLC: *t*<sub>r</sub> = 5.0 min (90 % acetonitrile in H<sub>2</sub>O), 91 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.98 (3H, s, CH<sub>3</sub>), 5.13 (1H, d, *J* = 16.3 Hz, ½CH<sub>2</sub>), 5.33 (1H, d, *J* = 16.3 Hz, ½CH<sub>2</sub>), 6.79-6.87 (3H, m, ArH), 7.00-7.11 (2H, m, ArH), 7.20-7.35 (4H, m, ArH), 7.46 (1H, td, *J* = 1.5, 7.4 Hz, ArH), 7.72 (1H, dd, *J* = 1.2, 7.9 Hz, ArH), 7.83 (1H, dd, *J* = 1.2, 8.2 Hz, ArH).

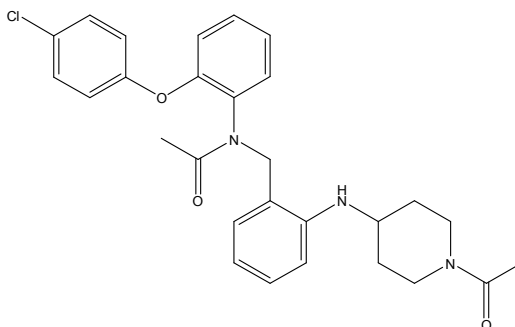
***N*-(2-Amino-benzyl)-*N*-[2-(4-chloro-phenoxy)-phenyl]-acetamide, 161,**

C<sub>21</sub>H<sub>19</sub>ClN<sub>2</sub>O<sub>2</sub>, MW 366.84,



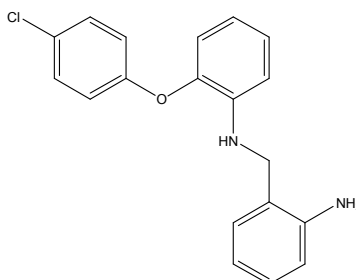
Using the general procedure for the reduction of the substituted 2-nitrobenzylalcohol the desired compound was obtained as a pale yellow solid, 53 mg, 62 % yield. R.f. 0.25 (1:1, DCM:EtOAc), <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.92 (3H, s, CH<sub>3</sub>), 4.55 (2H, br.s, NH<sub>2</sub>), 4.73-4.87 (2H, m, CH<sub>2</sub>), 6.38-6.52 (3H, m, ArH), 6.58-6.64 (2H, m, ArH), 6.77 (1H, d, *J* = 8.2 Hz, ArH), 6.96-7.05 (3H, m, ArH), 7.19-7.25 (3H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): δ 22.2 (CH<sub>3</sub>), 49.3 (CH<sub>2</sub>), 115.41, 116.7, 118.3 (ArCH), 119.8 (ArC), 120.7, 123.8, 129.3, 129.6, 129.9, 130.4, 131.9 (ArCH), 132.1, 146.5, 153.4, 154.4 (ArC), 171.7 (CO).

***N*-[2-(1-Acetyl-piperidin-4-ylamino)-benzyl]-*N*-[2-(4-chloro-phenoxy)-phenyl]-acetamide, 162**, C<sub>28</sub>H<sub>30</sub>ClN<sub>3</sub>O<sub>3</sub>, MW 492.01,



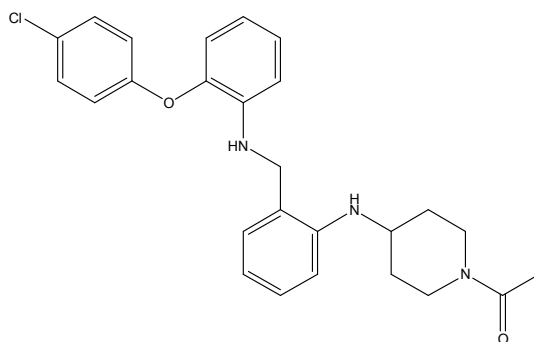
Using the general procedure for the reductive amination of the substituted diphenylether aniline with the substituted 2-acetamide benzaldehyde the desired compound was isolated as a cream oil, 45 mg, 63 % yield. R.f. 0.72 (10 % MeOH in EtOAc), LCMS:  $t_r$  = 5.4 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min),  $m/z$  M+H 492.49, HPLC:  $t_r$  = 6.42 min (90 % acetonitrile in H<sub>2</sub>O), 99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  (Multiple signals observed due to restricted rotation and therefore the presence of rotamers) 1.34-1.51 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 1.55-1.66 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 1.75-1.79 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 1.91, 1.93 (3H, s, CH<sub>3</sub>), 1.96-2.01 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 2.08, 2.09 (3H, s, CH<sub>3</sub>), 2.91-2.97 ( $\frac{1}{2}$ H, m,  $\frac{1}{4}$ CH<sub>2</sub>), 3.02-3.09 ( $\frac{1}{2}$ H, m,  $\frac{1}{4}$ CH<sub>2</sub>), 3.11-3.18 ( $\frac{1}{2}$ H, m,  $\frac{1}{4}$ CH<sub>2</sub>), 3.21-3.27 ( $\frac{1}{2}$ H, m,  $\frac{1}{4}$ CH<sub>2</sub>), 3.33-3.41 (1H, m, CH), 3.68-3.74 ( $\frac{1}{2}$ H, m,  $\frac{1}{4}$ CH<sub>2</sub>), 3.81-3.86 ( $\frac{1}{2}$ H, m,  $\frac{1}{4}$ CH<sub>2</sub>), 3.99-4.04 ( $\frac{1}{2}$ H, m,  $\frac{1}{4}$ CH<sub>2</sub>), 4.23-4.28 ( $\frac{1}{2}$ H, m,  $\frac{1}{4}$ CH<sub>2</sub>), 4.36 ( $\frac{1}{2}$ H, d,  $J$  = 14.4Hz,  $\frac{1}{4}$ CH<sub>2</sub>), 4.65 ( $\frac{1}{2}$ H, d,  $J$  = 14.8Hz,  $\frac{1}{4}$ CH<sub>2</sub>), 4.92 ( $\frac{1}{2}$ H, d,  $J$  = 14.4Hz,  $\frac{1}{4}$ CH<sub>2</sub>), 5.25 ( $\frac{1}{2}$ H, d,  $J$  = 14.4Hz,  $\frac{1}{4}$ CH<sub>2</sub>), 5.58-5.66 (1H, m, NH), 6.30-6.37 (3H, m, ArH), 6.44-6.50 (2H, m, ArH), 6.63-6.66 ( $\frac{1}{2}$ H, m, ArH), 6.72-6.74 ( $\frac{1}{2}$ H, m, ArH), 7.03-7.12 (3H, m, ArH), 7.13-7.16 (1H, m, ArH), 7.17-7.23 (2H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta$  21.5, 21.5, 22.0 (CH<sub>3</sub>), 30.6, 31.3, 31.9, 32.3, 39.5, 39.7, 44.7 (CH<sub>2</sub>), 48.4, 48.7 (CH), 49.5, 49.9 (CH<sub>2</sub>), 110.1, 110.2, 114.9, 115., 117.8, 118.1, 119.6, 119.7, 120.5, 120.9, 123.7, 123.8, 129.1, 129.1, 129.3, 129.4, 129.6, 129.7, 129.9 (ArCH), 131.6, 131.7 (ArC), 132.1, 132.2 (ArCH), 145.8, 145.9, 153.3, 153.8, 154.0, 154.3 (ArC), 168.7, 168.7, 171.6, 171.7 (CO). HRMS: Calcd for C<sub>28</sub>H<sub>30</sub>ClN<sub>3</sub>O<sub>3</sub> (M+H)<sup>+</sup> 492.2048, found (M+H)<sup>+</sup> 492.2049.

**[2-(4-Chloro-phenoxy)-phenyl]-(2-amino-benzyl)-amine, 163**, C<sub>19</sub>H<sub>17</sub>ClN<sub>2</sub>O, MW 324.80,



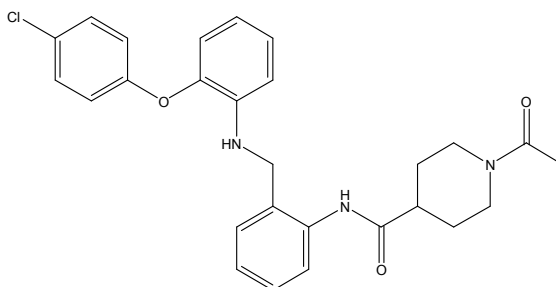
Using the general procedure for the reduction of the substituted 2-nitrobenzylalcohol the desired compound was obtained as a pale yellow solid, 118 mg, >100 % yield. R.f. 0.35 (EtOAc), m.p. 178-180 °C (from hexane), LCMS:  $t_r$  = 5.51 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min),  $m/z$  M-H 323.4, HPLC:  $t_r$  = 5.95 min (90 % acetonitrile in H<sub>2</sub>O), 85 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  3.99 (2H, s, NH<sub>2</sub>), 4.16 (1H, s, NH), 4.23 (2H, s, CH<sub>2</sub>), 6.66-6.77 (3H, m, ArH), 6.83-6.91 (4H, m, ArH), 7.07-7.16 (3H, m, ArH), 7.20-7.26 (2H, m, ArH).

**1-[4-(2-[2-(4-Chloro-phenoxy)-phenylamino]-methyl-phenylamino)-piperidin-1-yl]-ethanone, 164**, C<sub>26</sub>H<sub>28</sub>ClN<sub>3</sub>O<sub>2</sub>, MW 449.97,



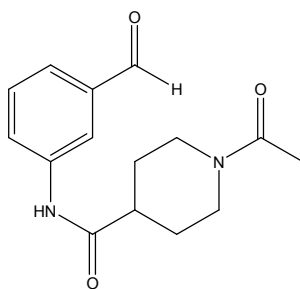
To a solution of [2-(4-chloro-phenoxy)-phenyl]-(2-amino-benzyl)-amine (50 mg, 0.15 mmol) and *N*-benzoyl-4-piperidone (0.038 mL, 0.30 mmol) in DCE (1.5 mL) was added acetic acid (0.03 mL) and sodium triacetoxyborohydride (82 mg, 0.38 mmol). The resulting reaction mixture was then subjected to microwave heating for 20 min at 140 °C.<sup>85</sup> A further portion of sodium triacetoxyborohydride (0.45 g, 0.2 mmol) was added and the solution was subjected to microwave heating for a further 10 min at 140 °C. NaHCO<sub>3</sub> was then added and the mixture was repeatedly extracted with DCM. The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The crude mixture was purified using flash chromatography (0-10 % MeOH in DCM) to afford the title compound as a cream oil, 23 mg, 33 % yield. R.f. 0.2 (1:1, EtOAc: Hexane), LCMS:  $t_r$  = 5.75 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min),  $m/z$  M+Na 472.41, HPLC:  $t_r$  = 6.19 min (90 % acetonitrile in H<sub>2</sub>O), 96 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  1.25 (2H, s, CH<sub>2</sub>), 1.83-1.92 (2H, m, CH<sub>2</sub>), 2.07 (3H, s, CH<sub>3</sub>), 2.98-3.04 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 3.11-3.17 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 3.47-3.51 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 3.56-3.62 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 4.09-4.17 (1H, m, NH), 4.21 (2H, td,  $J$  = 9.6 Hz, CH<sub>2</sub>NH), 4.70 (1H, s, NH), 6.65-6.71 (2H, m, ArH), 6.75 (1H, td,  $J$  = 1.6, 7.6 Hz, ArH), 6.81-6.86 (3H, m, ArH), 6.92 (1H, dd,  $J$  = 1.2, 8.0 Hz, ArH), 7.12 (1H, td,  $J$  = 1.2, 8.0 Hz, ArH), 7.15-7.17 (1H, m, ArH), 7.19-7.23 (3H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta$  21.5 (CH<sub>3</sub>), 29.7, 32.1, 39.7, 44.6 (CH<sub>2</sub>), 47.5 (CH<sub>2</sub>NH), 48.7 (CH), 110.9, 112.8, 116.8, 118.4, 119.4 (ArCH), 122.1 (ArC), 125.4 (ArCH), 127.7 (ArC), 129.2, 129.6, 130.4 (ArCH), 140.2, 143.3, 145.9, 156.1 (ArC), 168.8 (CO). HRMS: Calcd for C<sub>26</sub>H<sub>28</sub>ClN<sub>3</sub>O<sub>2</sub> (M+H)<sup>+</sup> 450.1943, found (M+H)<sup>+</sup> 450.1943.

**1-Acetyl-piperidine-4-carboxylic acid (2-[2-(4-chloro-phenoxy)-phenyl amino]-methyl-phenyl)-amide, 165**, C<sub>27</sub>H<sub>28</sub>ClN<sub>3</sub>O<sub>3</sub>, MW 477.98,



To a solution of [2-(4-chloro-phenoxy)-phenyl]-(2-amino-benzyl)-amine (48 mg, 0.15 mmol) and TEA (0.09 mL) in DCM (6 mL) at 0 °C, was added 1-acetylpiperidine-4-carboxyl chloride (58 mg, 0.6 mmol) and the resulting solution stirred was allowed to warm to room temperature and stirred for 24 h. NaHCO<sub>3</sub> was then added and the mixture was repeatedly extracted with DCM. The organic portions were then washed with 1M HCl. The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo*. The title compound was obtained as an off white solid, 44 mg, 62 % yield. R.f. 0.15 (10 % MeOH in DCM), m.p. 140-143 °C (from hexane), LCMS: *t*<sub>r</sub> = 1.25 min (95 % MeOH in water), *m/z* M-H 476.56 HPLC: *t*<sub>r</sub> = 1.71 min (90 % acetonitrile in H<sub>2</sub>O), 95 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 1.49-1.63 (2H, m, CH<sub>2</sub>), 1.74-1.83 (2H, m, CH<sub>2</sub>), 1.99 (3H, s, CH<sub>3</sub>), 2.18-2.24 (1H, m, CH), 2.49-2.57 (1H, m, CH<sub>2</sub>), 2.92-2.99 (1H, m, CH<sub>2</sub>), 3.69-3.73 (1H, m, CH<sub>2</sub>), 4.28 (3H, s, CH<sub>2</sub>NH and NH), 4.42-4.46 (1H, m, CH<sub>2</sub>), 6.77-6.86 (3H, m, ArH), 6.92-6.94 (1H, m, ArH), 7.06-7.10 (2H, m, ArH), 7.22-7.33 (5H, m, ArH), 8.05 (1H, d, *J* = 8.0 Hz, ArH), 8.85 (1H, s, NHCO). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 21.4 (CH<sub>3</sub>), 28.5, 28.7, 40.8 (CH<sub>2</sub>), 43.8 (CH), 45.6, 47.8 (CH<sub>2</sub>), 113.5, 118.7, 119.2, 119.7, 122.4, 124.5, 125.3 (ArCH), 127.2, 128.2 (ArC), 128.9, 129.7, 129.8 (ArCH), 137.4, 139.3, 144.0, 155.7 (ArC), 168.7, 172.1 (CO). HRMS: Calcd for C<sub>27</sub>H<sub>28</sub>ClN<sub>3</sub>O<sub>3</sub> (M+Na)<sup>+</sup> 500.1711, found (M+Na)<sup>+</sup> 500.1705.

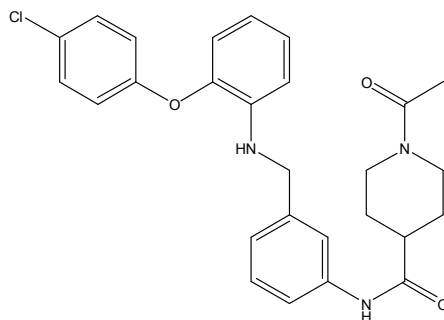
**1-Acetyl-piperidine-4-carboxylic acid (3-formyl-phenyl)-amide, 166**, C<sub>15</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub>, MW 274.32,



To a solution of 3-amino-benzaldehyde (200 mg, 1.65 mmol) and TEA (0.13 mL) in DCM (4 mL) at 0 °C, was added 1-acetylpiperidine-4-carboxyl chloride (0.6g, 3.3

mmol) and the resulting solution was allowed to warm to room temperature and stirred for 2 days.  $\text{NaHCO}_3$  was then added and the mixture was repeatedly extracted with DCM, the organic layers were then washed with HCl (1 M). The organic layers were combined, dried ( $\text{MgSO}_4$ ), filtered and evaporated *in vacuo*. The title compound was obtained as a cream oil, 97 mg, 22 % yield. R.f. 0.42 (10 % MeOH in DCM), LCMS:  $t_r$  = 0.99 min (95 % MeOH in water),  $m/z$  M-H 273.39, HPLC:  $t_r$  = 1.26 min (90 % acetonitrile in  $\text{H}_2\text{O}$ ), 81 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.66-1.90 (2H, m,  $\text{CH}_2$ ), 1.95-2.03 (2H, m,  $\text{CH}_2$ ), 2.11 (3H, s,  $\text{CH}_3$ ), 2.50-2.61 (1H, m, CH), 2.66-2.76 (1H, m,  $\frac{1}{2}\text{CH}_2$ ), 3.09-3.20 (1H, m,  $\frac{1}{2}\text{CH}_2$ ), 3.89-3.94 (1H, m,  $\frac{1}{2}\text{CH}_2$ ), 4.61-4.65 (1H, m,  $\frac{1}{2}\text{CH}_2$ ), 7.48 (1H, t,  $J$  = 7.9 Hz, ArH), 7.61 (1H, td,  $J$  = 1.2, 7.7 Hz, ArH), 7.90-8.00 (4H, m, ArH and NH), 9.97 (1H, s, CHO).

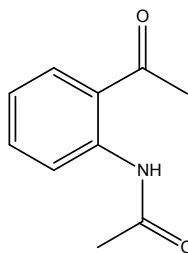
**1-Acetyl-piperidine-4-carboxylic acid (3-[2-(4-chloro-phenoxy)-phenyl amino]-methyl-phenyl)-amide, 167**,  $\text{C}_{27}\text{H}_{28}\text{ClN}_3\text{O}_3$ , MW 477.98,



Using the general procedure for the reductive amination of the substituted diphenylether aniline with the substituted 2-acetamide benzaldehyde the desired compound was isolated as a cream wax, 70 mg, 41 % yield. R.f. 0.22 (10 % MeOH in EtOAc), LCMS:  $t_r$  = 5.5 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min),  $m/z$  M-H 476.42, HPLC:  $t_r$  = 1.73 min (90 % acetonitrile in  $\text{H}_2\text{O}$ ), 93 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.63-1.77 (2H, m,  $\text{CH}_2$ ), 1.78-1.90 (2H, m,  $\text{CH}_2$ ), 2.09 (3H, s,  $\text{CH}_3$ ), 2.38-2.50 (1H, m, CH), 2.63-2.73 (1H, m,  $\frac{1}{2}\text{CH}_2$ ), 3.11 (1H, td,  $J$  = 2.7, 13.9 Hz,  $\frac{1}{2}\text{CH}_2$ ), 3.88 (1H, d,  $J$  = 13.6 Hz,  $\frac{1}{2}\text{CH}_2$ ), 4.33 (2H, s,  $\text{CH}_2$ ), 4.58-4.63 (2H, m,  $\frac{1}{2}\text{CH}_2$  and NH), 6.60-6.66 (2H, m, ArH), 6.82- (1H, dd,  $J$  = 1.5, 8.4 Hz, ArH), 6.86-6.92 (2H, m, ArH), 6.95-7.06 (2H, m, ArH), 7.21-7.28 (3H, m, ArH), 7.39-7.44 (3H, m, ArH and NH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz):  $\delta$  21.6 ( $\text{CH}_3$ ), 28.6, 28.9, 41.0, (CH<sub>2</sub>), 44.1 (CH), 45.8, 47.6 (CH<sub>2</sub>), 112.0, 117.2, 118.5, 118.7, 118.8, 119.4, 123.2, 125.4 (ArCH), 127.7 (ArC), 129.4, 129.8 (ArCH), 129.8, 138.1, 140.2, 140.5 (ArC), 169.0, 172.3 (CO). HRMS: Calcd for  $\text{C}_{27}\text{H}_{28}\text{ClN}_3\text{O}_3$  (M+H)<sup>+</sup> 478.1892, found (M+H)<sup>+</sup> 478.1878.

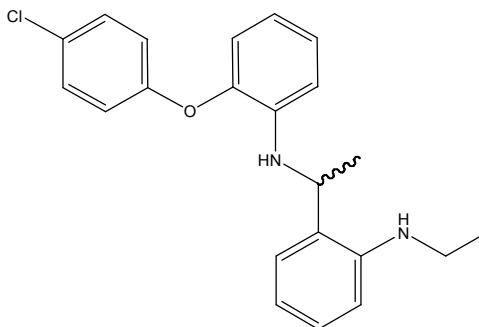


***N*-(2-Acetyl-phenyl)-acetamide, 168**, C<sub>10</sub>H<sub>11</sub>NO<sub>2</sub>, MW 177.20,



A solution of 2-aminoacetophenone (2.0 g, 14.8 mmol) in DCM (80 mL) was cooled to 0 °C, to this was added TEA (2.4 mL) and acetyl chloride (2.06 mL, 30 mmol). The resulting solution was stirred at r.t. for 30 min. NaHCO<sub>3</sub> was added and the solution was extracted, the organic layers were then washed with HCl (1M) and brine. The organic layers were dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo* to yield the desired product as a brown solid, 2.3 g, 89 % yield. R.f. 0.49 (EtOAc), m.p. 68-70 °C, LCMS: *t*<sub>r</sub> = 1.32 min (80 % MeOH in water), *m/z* M-H 175.79, HPLC: *t*<sub>r</sub> = 1.60 min (90 % acetonitrile in H<sub>2</sub>O), 94 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 2.15 (3H, s, CH<sub>3</sub>), 2.58, (3H, s, CH<sub>3</sub>), 6.99-7.06 (1H, m, ArH), 7.43-7.49 (1H, m, ArH), 7.80 (1H, dd, *J* = 1.5, 8.15 Hz, ArH), 8.65 (1H, dd, *J* = 1.0, 8.4 Hz, ArH), 11.63 (1H, br.s, NH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): δ 25.7, 28.8 (CH<sub>3</sub>), 120.8 (ArCH), 121.7 (ArC), 122.4, 131.7, 135.3 (ArCH), 141.1 (ArC), 169.6, 202.9 (CO). HRMS: Calcd for C<sub>10</sub>H<sub>11</sub>NO<sub>2</sub> (M+H)<sup>+</sup> 178.0863, found (M+H)<sup>+</sup> 178.0858.

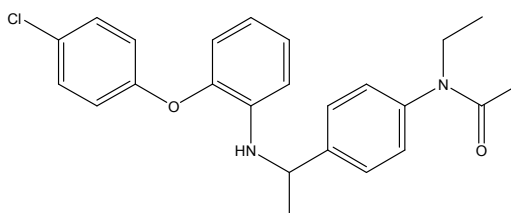
***N*-(2-1-[2-(4-Chloro-phenoxy)-phenylamino]-ethyl-phenyl)-ethylamine, 169**,  
C<sub>22</sub>H<sub>23</sub>ClN<sub>2</sub>O, MW 366.88,



A solution of 2-(4-chloro-phenoxy)-phenylamine (298 mg, 1.4 mmol), *N*-(2-acetyl-phenyl)-acetamide, (200 mg, 1.13 mmol) and chlorotriisopropoxytitanium IV (0.53 mL, 2.26 mmol) in toluene (15 mL) was stirred at r.t. for 4 days. NaHCO<sub>3</sub> was added and the mixture was extracted repeatedly with EtOAc, dried (MgSO<sub>4</sub>) and evaporated to dryness. The residue was re-dissolved in THF (20 mL) and cooled to 0 °C, to this was added succinic acid (270 mg, 2.26 mmol) and borane (1M in THF, 2.3 mL, 2.26 mmol). The reaction was slowly warmed to r.t. and stirred for 8 h.<sup>96</sup> NaHCO<sub>3</sub> was added and the volatile solvents removed *in vacuo*, the mixture was then extracted with EtOAc and dried (MgSO<sub>4</sub>). The crude material was purified by flash chromatography (0-100 % DCM in hexane) to yield the product as an off white oil, 79 mg, 19 % yield. LCMS: *t*<sub>r</sub> =

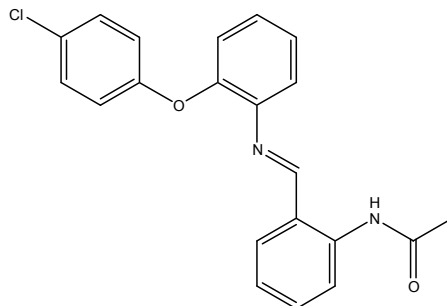
1.42 min (95 % MeOH in water),  $m/z$  M-H 365.33, HPLC:  $t_r$  = 4.49 min (90 % acetonitrile in water), 97 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.17 (3H, t,  $J$  = 7.2 Hz,  $\text{CH}_3\text{CH}_2$ ), 1.56 (3H, d,  $J$  = 6.7 Hz,  $\text{CH}_3\text{CH}$ ), 3.10 (2H, q,  $J$  = 14.1 Hz,  $\text{CH}_2$ ), 4.22 (1H, d,  $J$  = 6.0 Hz, NH), 4.53 (1H, q,  $J$  = 13.3 Hz, CH), 4.59 (1H, br.s, NH), 6.66-6.93 (7H, m, ArH), 7.01 (1H, td,  $J$  = 7.9, 1.5 Hz, ArH), 7.16-7.31 (4H, m, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz): 14.9, 19.9 ( $\text{CH}_3$ ), 38.1 ( $\text{CH}_2$ ), 50.9 (CH), 111.1, 113.6, 117.0, 118.0, 118.6, 119.5, 125.5, 126.5 (ArCH), 128.6, 127.8 (ArC), 128.3, 129.7 (ArCH), 13.7, 143.3, 146.7, 156.4 (ArC). HRMS: Calcd for  $\text{C}_{22}\text{H}_{23}\text{ClN}_2\text{O}$  ( $\text{M}+\text{Na}$ ) $^+$  389.1386, found ( $\text{M}+\text{Na}$ ) $^+$  389.1391.

***N*-(4-(1-[2-(4-Chloro-phenoxy)-phenylamino]-ethyl)-phenyl)-*N*-ethyl-acetamide, 170**,  $\text{C}_{24}\text{H}_{25}\text{ClN}_2\text{O}_2$ , MW 408.92,



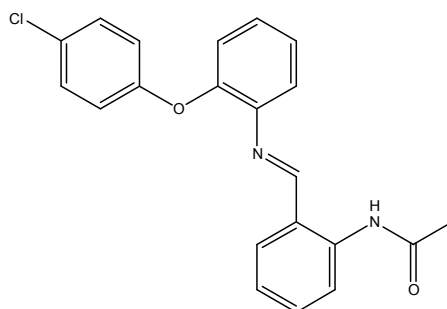
*N*-(4-(1-[2-(4-Chloro-phenoxy)-phenylamino]-ethyl)-phenyl)-ethane (50 mg, 0.14 mmol) was dissolved in DCM (1 mL) and cooled to 0°C, to this was added acetyl chloride (0.04 mL, 0.56 mmol) and TEA (0.02 mL, 0.42 mmol). This was then allowed to warm to room temperature and stirred for 1h. Saturated  $\text{NaHCO}_3$  solution was added and the mixture was extracted with DCM, dried ( $\text{MgSO}_4$ ) and purified by flash chromatography to yield the title compound as an off-white oil, 23 mg, 40 % yield. R.f. 0.55 (EtOAc), LCMS:  $t_r$  = 1.37 min (95 % MeOH in water),  $m/z$  M-H 407.34, HPLC:  $t_r$  = 3.03 min (90 % acetonitrile in  $\text{H}_2\text{O}$ ), 96 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.15 (3H, dt,  $J$  = 6.8, 10.4 Hz,  $\text{CH}_3\text{CH}_2$ ), 1.43 (3H, dd,  $J$  = 6.4, 15.6 Hz,  $\text{CH}_3\text{CH}$ ), 1.74 (3H, d,  $J$  = 25.6 Hz,  $\text{CH}_3\text{CO}$ ), 3.07-3.15 (1H, m,  $\frac{1}{2}\text{CH}_2$ ), 4.24-4.36 (2H, m,  $\frac{1}{2}\text{CH}_2$  and NH), 4.64-4.75 (1H, m, CH), 6.51 (1H, dd,  $J$  = 1.6, 8.4 Hz, ArH), 6.57-6.67 (2H, m, ArH), 6.74-6.84 (2H, m, ArH), 6.86-6.90 (1H, m, ArH), 6.92-6.97 (1H, m, ArH), 7.06 (1H, td,  $J$  = 1.6, 8.0 Hz, ArH), 7.21-7.35 (4H, m, ArH), 7.39-7.47 (1H, m, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz): 12.8, 22.5, 23.1 ( $\text{CH}_3$ ), 43.5 ( $\text{CH}_2$ ), 47.5 (CH), 112.7, 117.7, 118.8, 119.4, 125.3, 126.9, 128.3, 129.4, 129.7, 130.3 (ArCH), 138.9, 139.9, 141.7, 142.4, 143.1, 156.3 (ArC), 170.5 (CO). HRMS: Calcd for  $\text{C}_{24}\text{H}_{25}\text{ClN}_2\text{O}_2$  ( $\text{M}+\text{Na}$ ) $^+$  431.1497, found ( $\text{M}+\text{Na}$ ) $^+$  431.1487.

***N*-(2-([2-(4-Chloro-phenoxy)-phenylimino]-methyl)-phenyl)-acetamide, 171,**  
 $C_{21}H_{17}ClN_2O_2$ , MW 364.82, (Method 1),



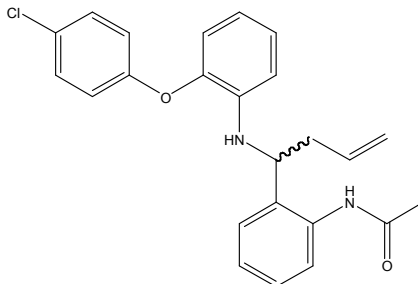
A solution of 2-(4-chloro-phenoxy)-phenylamine (100 mg, 0.46 mmol) and *N*-(2-formyl-phenyl)-acetamide (74 mg, 0.46 mmol) in anhydrous DCM (5mL) was stirred at r.t. and to this was added  $MgSO_4$  (550 mg, 4.6 mmol) and the resulting mixture stirred for a further 18 h at r.t. The mixture was then filtered and the solid was washed with DCM. The filtrate was then evaporated to dryness to yield the desired product as an oil. The product was identified by NMR, as no CHO peak was visible in the  $^1H$  NMR and it had been replaced with an imine peak. The product was used crude in all following experiments. A small sample was purified for analytical purposes.  $^1H$  NMR ( $CDCl_3$ , 270 MHz):  $\delta$  2.03 (3H, s,  $CH_3$ ), 6.87-7.46 (11H, m, ArH), 8.60 (1H, s,  $N=CH$ ), 8.72 (1H, d,  $J = 8.5$  Hz, ArH).  $^{13}C$  NMR ( $CDCl_3$ , 101 MHz): 24.9 ( $CH_3$ ), 116.7, 119.2, 119.6, 120.3 (ArCH), 120.6 (ArC), 122.5, 125.0, 127.9 (ArCH), 128.2 (ArC), 129.8, 132.7 (ArCH), 140.4, 141.7, 149.6, 156.1 (ArC), 163.4 (CH), 169.9 (CO).

***N*-(2-([2-(4-Chloro-phenoxy)-phenylimino]-methyl)-phenyl)-acetamide, 171,**  
 $C_{21}H_{17}ClN_2O_2$ , MW 364.82, (Method 2),<sup>95</sup>



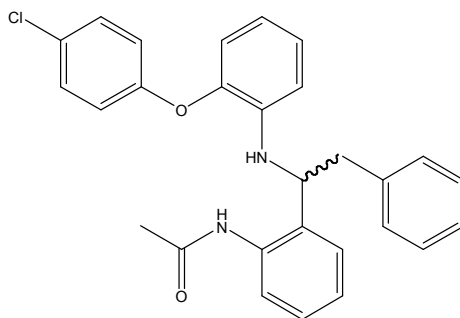
A solution of 2-(4-chloro-phenoxy)-phenylamine (100 mg, 0.46 mmol) and *N*-(2-formyl-phenyl)-acetamide (74 mg, 0.46 mmol) in anhydrous DCM (5mL) was stirred at r.t. and to this was added  $TiCl(O^iPr)_3$  (0.25 mL, 1 mmol). The resulting mixture was stirred for a further 4 h at room temperature. The mixture was then evaporated to dryness to yield the desired product as an oil. As in Method 1 (see above) the product could easily be identified by  $^1H$  NMR. The product was used crude in all subsequent experiments.

***N*-(2-(1-[2-(4-Chloro-phenoxy)-phenylamino]-but-2-enyl)-phenyl)-acetamide, 172,**  
 $C_{24}H_{23}ClN_2O_2$ , MW 406.90,



*N*-(2-([2-(4-Chloro-phenoxy)-phenylimino]-methyl)-phenyl)-acetamide (500 mg, assumed 100 % pure, 1.4 mmol) was dissolved in THF (15 mL) and cooled to 0 °C under a  $N_2$  atmosphere, to this was added  $BF_3OEt_2$  (0.18 mL, 1.4 mmol) and allylmagnesium bromide (1 M in ether, 4.2 mL, 4.2 mmol). The resulting solution was stirred at r.t. for 18 h. The reaction was then quenched with sat.  $NH_4Cl$  solution then extracted with EtOAc and dried ( $MgSO_4$ ). The crude product was purified by flash chromatography (0-50 % EtOAc in hexane) to yield the desired product as a light brown solid, 320 mg, 57 % yield. R.f. 0.36 (DCM), LCMS:  $t_r$  = 1.59 min (95 % MeOH in water),  $m/z$   $M^+H$  (+Na) 429.13,  $M+H$  407.15, HPLC:  $t_r$  = 2.45min (90 % acetonitrile in water), 92 %, HRMS: Calcd for  $C_{24}H_{23}ClN_2O_2$  ( $M+H$ )<sup>+</sup> 407.1521, found ( $M+H$ )<sup>+</sup> 407.1503.  $^1H$  NMR ( $CDCl_3$ , 400 MHz):  $\delta$  1.86 (3H, s,  $CH_3$ ), 2.54-2.62 (2H, m,  $CH_2$ ), 4.31 (1H, t,  $J$  = 6.4 Hz,  $CHNH$ ), 4.60 (1H, s, NH), 5.10 (1H, s,  $\frac{1}{2}CH_2CH$ ), 5.13 (1H, d,  $J$  = 5.2 Hz,  $\frac{1}{2}CH_2CH$ ), 5.65-5.75 (1H, m,  $CHCH_2$ ), 6.66 (1H, d,  $J$  = 8.0 Hz, ArH), 6.77 (1H, t,  $J$  = 8.0 Hz, ArH), 6.87-6.96 (4H, m, ArH), 7.13 (1H, t,  $J$  = 7.6 Hz, ArH), 7.26-7.31 (4H, m, ArH), 8.05 (1H, d,  $J$  = 8.4 Hz, ArH), 9.40 (1H, br.s,  $NHCO$ ).  $^{13}C$  NMR ( $CDCl_3$ , 101 MHz): 24.3 ( $CH_3$ ), 40.6 (CH), 58.9 ( $CH_2$ ), 114.9, 118.0 (ArCH), 119.3 ( $CH_2$ ), 119.8, 120.0, 123.1, 124.7, 125.6 (ArCH), 128.0 (ArC), 128.2, 128.2, 129.8 (ArCH), 130.7 (ArC), 134.0 (CH), 136.9, 139.3, 143.5, 156.1 (ArC), 168.1 (CO).

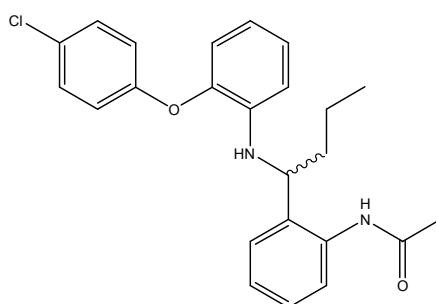
***N*-(2-(1-[2-(4-Chloro-phenoxy)-phenylamino]-2-phenyl-ethyl)-phenyl)-acetamide, 173,** MW 456.96,



*N*-(2-([2-(4-Chloro-phenoxy)-phenylimino]-methyl)-phenyl)-acetamide (111 mg, assumed 100 % pure, 0.3 mmol) was dissolved in THF (5 mL) and cooled to 0 °C under a  $N_2$  atmosphere, to this was added  $BF_3OEt_2$  (0.04 mL, 0.3 mmol) and

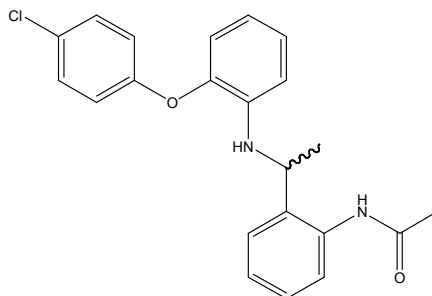
benzylmagnesium bromide (2 M in THF, 0.3 mL, 1.2 mmol). The resulting solution was stirred at r.t. for 18 h. The reaction was then quenched with sat.  $\text{NH}_4\text{Cl}$  solution, extracted with EtOAc and dried ( $\text{MgSO}_4$ ). The crude product was purified by flash chromatography (0-20 % EtOAc in DCM) to yield the desired product as an oil, 20 mg, 14 % yield. R.f. 0.25 (DCM), LCMS:  $t_r = 1.26$  min (95 % MeOH in water),  $m/z$  M $^+$ H 455.15, HPLC:  $t_r = 2.73$  min (90 % acetonitrile in water), 97 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.82 (3H, s,  $\text{CH}_3$ ), 3.02-3.04 (2H, m,  $\text{CH}_2$ ), 4.43 (1H, t,  $J = 8.0$  Hz, CH), 4.50 (1H, br.s, NH), 6.52 (1H, d,  $J = 6.8$  Hz, ArH), 6.63-6.74 (4H, m, ArH), 6.79-6.84 (1H, m, ArH), 6.98-7.00 (1H, m, ArH), 7.05 (1H, t,  $J = 7.2$  Hz, ArH), 7.15-7.24 (7H, m, ArH), 7.91 (1H, d,  $J = 8.4$  Hz, ArH), 8.90 (1H, br.s,  $\text{NHCO}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta$  24.3 ( $\text{CH}_3$ ), 42.9 ( $\text{CH}_2$ ), 60.8 (CH), 114.9, 117.9, 119.6, 119.7, 123.4, 124.9, 125.6, 127.2 (ArCH), 127.9 (ArC), 128.1, 128.2, 128.9, 129.1, 129.8 (ArCH), 131.2, 136.7, 136.8, 139.3, 143.4, 156.0 (ArC), 168.2 (CO). HRMS: Calcd for  $\text{C}_{28}\text{H}_{25}\text{ClN}_2\text{O}_2$  (M+H) $^+$  457.1677, found (M+H) $^+$  457.1666.

***N*-(2-(1-[2-(4-chloro-phenoxy)-phenylamino]-butyl)-phenyl)-acetamide, 174,**  
 $\text{C}_{24}\text{H}_{25}\text{ClN}_2\text{O}_2$ , MW 408.92,



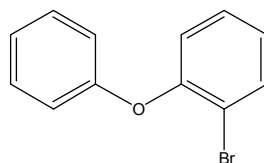
To a solution of *N*-(2-(1-[2-(4-chloro-phenoxy)-phenylamino]-but-2-enyl)-phenyl)-acetamide, (50 mg, 0.12 mmol) in EtOAc (25 mL) was added Pd/C (15 mg). The solution was then stirred under a  $\text{H}_2$  atmosphere for 15 min and filtered through Celite. Purification by flash chromatography (0-50 % EtOAc in hexane) afforded the desired product, 44 mg, 88 % yield. R.f. 0.42 (EtOAc), LCMS:  $t_r = 3.72$  min (90 % MeOH in water),  $m/z$  M+H 409.00, HPLC:  $t_r = 4.69$  min (90 % MeOH in water), 99 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  0.90 (3H, t,  $J = 7.6$  Hz,  $\text{CH}_2\text{CH}_3$ ), 1.21-1.39 (2H, m,  $\text{CH}_2$ ), 1.79-1.86 (2H, m,  $\text{CH}_2$ ), 1.87 (3H, s,  $\text{CH}_3\text{CO}$ ), 4.29 (1H, t,  $J = 7.6$  Hz, CH), 4.37 (1H, br.s, NH), 6.70 (1H, d,  $J = 8.0$  Hz, ArH), 6.73-6.78 (1H, m, ArH), 6.85-6.87 (1H, m, ArH), 6.90-6.96 (3H, m, ArH), 7.11 (1H, t,  $J = 7.2$  Hz, ArH), 7.25-7.31 (4H, m, ArH), 8.05 (1H, d,  $J = 8.0$  Hz, ArH), 9.36 (1H, br.s,  $\text{NHCO}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz): 13.7 ( $\text{CH}_3\text{CH}_2$ ), 19.6 ( $\text{CH}_2$ ), 24.4 ( $\text{CH}_3\text{CO}$ ), 38.0 ( $\text{CH}_2$ ), 60.0 (CH), 114.7, 118.3, 119.4, 119.5, 123.0, 124.4, 125.4, 127.9, 128.1, 128.5, 129.9 (ArCH), 130.9, 136.8, 139.3, 143.7, 156.0 (ArC), 168.1 (CO). HRMS: Calcd for  $\text{C}_{24}\text{H}_{25}\text{ClN}_2\text{O}_2$  (M+H) $^+$  409.1677, found (M+H) $^+$  409.1677.

***N*-(2-(1-[2-(4-Chloro-phenoxy)-phenylamino]-ethyl)-phenyl)-acetamide, 175,**  
 $C_{22}H_{21}ClN_2O_2$ , MW 380.87,



A cerium chloride suspension was prepared, based upon methods described by Lui<sup>103</sup> and Imamoto<sup>104</sup>.  $CeCl_3 \cdot 7H_2O$  (stored in the oven, 515 mg, 1.38 mmol) was heated under high-vac for 15 min and allowed to cool to r.t. then 0 °C in an ice bath. To this was added THF (3 mL) and methylmagnesium bromide (3 M in diethyl ether, 0.46 mL, 1.38 mmol), this was then stirred at r.t. for 2 h. To this was added *N*-(2-([2-(4-chloro-phenoxy)-phenylimino]-methyl)-phenyl)-acetamide intermediate (166 mg, 0.46 mmol) and stirred at r.t. for a further 18 h.  $NaHCO_3$  was added and the mixture was extracted with EtOAc, dried ( $MgSO_4$ ) and purified by flash chromatography (0-100 % DCM) to yield the desired product as an off white oil, 14 mg, 8 % yield. R.f. 0.56 (DCM with TEA), LCMS:  $t_r$  = 1.08 min (90 % MeOH in water),  $m/z$   $M+Na$  403.20, HPLC:  $t_r$  = 2.6 min (90 % acetonitrile in water), 94 %,  $^1H$  NMR ( $CDCl_3$ , 270 MHz):  $\delta$  1.55 (3H, d,  $J$  = 6.6 Hz,  $CH_3CH$ ), 1.90 (3H, s,  $CH_3CO$ ), 4.25 (1H, d,  $J$  = 3.0 Hz,  $CHNH$ ), 4.52-4.54 (1H, m, CH), 6.73-6.78 (2H, m, ArH), 6.84-6.99 (4H, m, ArH), 7.11 (1H, t,  $J$  = 7.7 Hz, ArH), 7.24-7.31 (4H, m, ArH), 8.02 (1H, d,  $J$  = 8.0 Hz, ArH), 9.16 (1H, br.s, NH).  $^{13}C$  NMR ( $CDCl_3$ , 101 MHz): 21.5, 24.4 ( $CH_3$ ), 53.9 (CH), 114.6, 118.5, 119.4, 119.6, 123.2, 124.7, 125.4, 127.3, 128.1 (ArCH), 128.2 (ArC), 129.9 (ArCH), 132.0, 136.8, 138.9, 143.9, 155.9 (ArC), 168.2 (CO). HRMS: Calcd for  $C_{22}H_{21}ClN_2O_2$  ( $M+H$ )<sup>+</sup> 381.1364, found ( $M+H$ )<sup>+</sup> 381.1352.

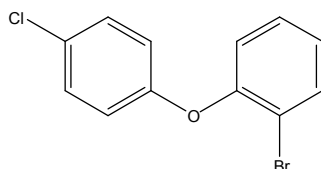
**1-Bromo-2-phenoxy-benzene, 176,**  $C_{12}H_9BrO$ , MW 249.10,



The above compound was prepared using a method by Nie *et al.*<sup>107</sup> A mixture of 2-bromophenol (0.211 mL, 2 mmol), phenylboronic acid (490 mg, 4 mmol), copper acetate (364 mg, 2 mmol), TEA (1.38 mL, 10 mmol) and 4Å molecular sieves in DCM (25 mL) was stirred at r.t. for 18 h.<sup>107</sup> The slurry was filtered through Celite and concentrated *in vacuo*. This was then diluted with EtOAc and  $NaHCO_3$  solution, extracted and the organic portions were washed with brine and dried ( $MgSO_4$ ). The crude mixture was purified by flash chromatography (hexane) to yield the desired

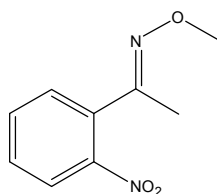
product as a colourless oil, 232 mg, 47 % yield. R.f. 0.75 (DCM), LCMS:  $t_r$  = 1.3 min (95 % MeOH in water),  $m/z$  M-H 246.84, 248.86, HPLC:  $t_r$  = 2.88 min (90 % acetonitrile in water), 98%,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  6.95-7.04 (4H, m, ArH), 7.11 (1H, td,  $J$ =1.1, 8.0 Hz, ArH), 7.22-7.37 (3H, m, ArH), 7.61-7.65 (1H, m, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz): 115.0 (ArC), 118.2, 120.7, 123.5, 125.1, 128.8, 129.9, 133.9 (ArCH), 153.8, 156.9 (ArC).

**1-Bromo-2-phenoxy-4'chlorobenzene, 177**,  $\text{C}_{12}\text{H}_8\text{BrClO}$ , MW 283.55,



The above compound was prepared using a method by Nie *et al.*<sup>107</sup> A mixture of 2-bromophenol (0.2 mL, 2 mmol), phenylboronic acid (600 mg, 4 mmol), copper acetate (350 mg, 2 mmol), TEA (1.4 mL, 10 mmol) and powdered 4Å molecular sieves (~ 2 g) in DCM (25 mL) was stirred at r.t. for 18 h. The slurry was filtered through Celite and concentrated *in vacuo*. This was then diluted with EtOAc and  $\text{NaHCO}_3$  solution, extracted and the organic portions were washed with brine and dried ( $\text{MgSO}_4$ ). The crude mixture was purified by flash chromatography (hexane) to yield the desired product as a colourless oil, 320 mg, 59 % yield. R.f. 0.72 (DCM), HPLC:  $t_r$  = 3.29 min (90 % acetonitrile in water), >99 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  6.85-6.91 (1H, m, ArH), 6.96 (1H, dd,  $J$  = 1.4, 8.0Hz, ArH), 7.00-7.07 (1H, m, ArH), 7.25-7.29 (3H, m, ArH), 7.62 (1H, dd,  $J$  = 1.6, 8.0Hz, ArH).

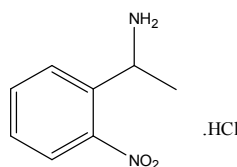
**1-(2-Nitro-phenyl)-ethanone-*O*-methyl-oxime, 178**,  $\text{C}_9\text{H}_9\text{N}_2\text{O}_3$ , MW 194.19,



The above compound was synthesised using a method as described by Salerno *et al.*<sup>111</sup> To a solution of 2-nitroacetophenone (1.9 g, 10.9 mmol), methoxyamine hydrochloride (0.96 g, 10.9 mmol) in anhydrous pyridine (38 mL) and anhydrous EtOH (38 mL) was added powdered 4Å molecular sieves (~1 g). The resulting mixture was heated at reflux for 3h. The resulting mixture was filtered through Celite to remove the molecular sieves and then evaporated to dryness. The solid was re-dissolved in EtOAc and extracted with 20 %  $\text{NaHCO}_3$  solution, this was then dried ( $\text{MgSO}_4$ ) and evaporated *in vacuo* to yield the desired compound as a mixture of enantiomers, yellow oil, 1.95 g, 87 % yield. The product was used crude in following reactions. R.f. 0.55 (DCM), HPLC:  $t_r$  = 1.87 min, 58 %,  $t_r$  = 2.39 min, 29 % (90 % acetonitrile in water), LCMS:  $t_r$  = 3.30

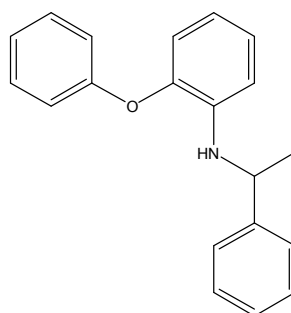
min,  $m/z$   $M^+H$  195.4,  $t_r$  = 4.00 min,  $m/z$   $M^+H$  195.3 (70 % MeOH in water).  $^1H$  NMR ( $CDCl_3$ , 400 MHz): Major isomer:  $\delta$  2.54 (3H, s,  $CH_3$ ), 3.69 (3H, s,  $OCH_3$ ), 7.23-8.08 (4H, m, ArH). Minor isomer:  $\delta$  2.14 (3H, s,  $CH_3$ ), 3.94 (3H, s,  $OCH_3$ ), 7.23-8.08 (4H, m, ArH).

**1-(2-Nitro-phenyl)-ethylamine hydrochloride, 179**,  $C_8H_{11}ClN_2O_2$ , MW 202.64,<sup>111</sup>



The above compound was synthesised using a method as described by Salerno *et al.*<sup>111</sup> A solution of 1-(2-nitro-phenyl)-ethanone-*O*-methyl-oxime (1.95 g, 10.05 mmol) in THF (7 mL) was cooled to 0 °C, to this was added borane/ THF complex (28 mL, 28.1 mmol) and the resulting solution was then heated at reflux for 6 h. The reaction was then cooled to -20 °C and water (2 mL) was added slowly followed by aq. 20 % KOH solution (2 mL) over 20 min. The resulting mixture was then heated at reflux for a further 2 h and then poured into DCM. The mixture was then extracted with brine and dried ( $MgSO_4$ ). To form the salt, the product was re-dissolved in DCM and then concentrated HCl (1.5 mL) was added and the mixture was stirred for 1 h. The resulting solid was removed by filtration and washed with ether and dried, 345 mg, 17 % yield. R.f. 0.32 (Hexane: DCM, 1:1), LCMS:  $t_r$  = 1.33 min (70 % MeOH in water),  $m/z$   $M+H$  167.2 (free base), HPLC:  $t_r$  = 2.09 min (90 % acetonitrile in water), >99 %,  $^1H$  NMR ( $CDCl_3$ , 400 MHz):  $\delta$  1.60 (3H, d,  $J$  = 6.8 Hz,  $CH_3$ ), 4.77-4.80 (1H, m, CH), 7.63-7.67 (1H, m, ArH), 7.86 (1H, td,  $J$  = 1.2, 7.6 Hz, ArH), 8.03-8.05 (2H, m, ArH), 8.72 (2H, br.s,  $NH_2$ ).

**(2-Phenoxy-phenyl)-(1-phenyl-ethyl)-amine, 180**,  $C_{20}H_{19}NO$ , MW 289.37,

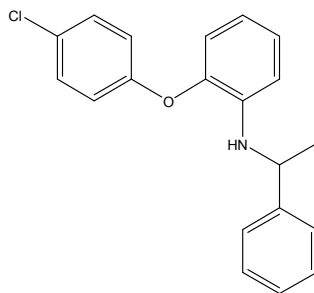


The above compound was synthesised using a modified method from Harris *et al.*<sup>113</sup> Palladium acetate (19 mg, 10 mol%) and rac-BINAP (51 mg, 10 mol%) were placed into an oven-dried flask, this was then evacuated and back filled with  $N_2$ . To this was then added (via syringe) 1-phenylethylamine (100 mg, 0.83 mmol), 1-bromo-2-phenoxy-benzene (185 mg, 0.74 mmol) and toluene (1 mL). This was stirred for 10 min at r.t. Sodium *t*-butoxide (95 mg, 1 mmol) and further toluene (1 mL) were then added.



The resulting solution was heated to reflux for 18 h. The mixture was then filtered through Celite and purified by flash chromatography (0-50 % DCM in hexane) to yield the desired product as an off white oil, 104 mg, 49 % yield. R.f. 0.45 (1:1, DCM: Hexane), LCMS:  $t_r$  = 2.18 min (90 % MeOH in water),  $m/z$  M+H 290.10, HPLC:  $t_r$  = 3.11 min (90 % acetonitrile in water), 98 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.47 (3H, d,  $J$  = 6.3 Hz,  $\text{CH}_3$ ), 4.45-4.58 (2H, m, CH and NH), 6.46 (1H, dd,  $J$  = 1.1, 8.0 Hz, ArH), 6.56 (1H, td,  $J$  = 1.4, 7.9 Hz, ArH), 6.80-6.89 (2H, m, ArH), 6.99-7.11 (3H, m, ArH), 7.18-7.36 (7H, m, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz): 25.2 ( $\text{CH}_3$ ), 53.3 (CH), 112.7, 116.7, 117.6, 119.2, 122.8, 124.8, 125.9, 126.9, 128.7, 129.8 (ArCH), 139.6, 143.0, 145.2, 157.7 (ArC). HRMS: Calcd for  $\text{C}_{20}\text{H}_{19}\text{NO}$  ( $\text{M}+\text{H}$ ) $^+$  290.1539, found ( $\text{M}+\text{H}$ ) $^+$  290.1529.

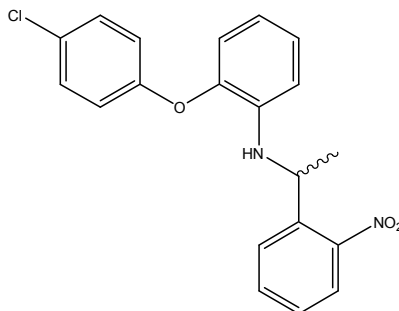
**[2-(4-Chloro-phenoxy)-phenyl]-(1-phenyl-ethyl)-amine, 181**,  $\text{C}_{20}\text{H}_{18}\text{ClNO}$ , MW 323.82,



The above compound was synthesised using a modified method from Harris *et al.*<sup>113</sup> Palladium acetate (10 mg, 10 mol%) and rac-BINAP (26 mg, 10 mol%) were placed into an oven dried flask, this was evacuated and back filled with  $\text{N}_2$ . To this was then added (via syringe) 1-phenylethylamine (50 mg, 0.42 mmol), 1-bromo-2-phenoxy-4-chlorobenzene (105 mg, 0.38 mmol) and toluene (1 mL). This was stirred for 10min at r.t. Sodium *t*-butoxide (50 mg, 0.5 mmol) and a further portion of toluene (1 mL) was then added. The resulting solution was heated to reflux for 3 h.<sup>113</sup> This was then filtered through Celite and purified by flash chromatography (hexane) to yield the desired product as a white solid, 51 mg, 41 % yield. R.f. 0.35 (hexane), m.p. 84-86 °C, LCMS:  $t_r$  = 4.67 min (90 % MeOH in water),  $m/z$  M-H 322.3, HPLC:  $t_r$  = 4.30 min (90 % acetonitrile in water), 97%,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.48 (3H, d,  $J$  = 6.0 Hz,  $\text{CH}_3$ ), 4.49-4.53 (2H, m, CH and NH), 6.48 (1H, t,  $J$  = 8.0 Hz, ArH), 6.92-6.95 (2H, m, ArH), 7.20-7.31 (7H, m, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz): 25.2 ( $\text{CH}_3$ ), 53.3 (CH), 113.0, 116.9, 118.8, 119.3, 125.3, 125.9, 127.1 (ArCH), 127.8 (ArC), 128.8, 129.8 (ArCH), 139.5, 142.6, 145.1, 156.4 (ArC). HRMS: Calcd for  $\text{C}_{20}\text{H}_{18}\text{ClNO}$  ( $\text{M}+\text{H}$ ) $^+$  324.1150, found ( $\text{M}+\text{H}$ ) $^+$  324.1136.

**[2-(4-Chloro-phenoxy)-phenyl]-[1-(2-nitro-phenyl)-ethyl]-amine, 182,**

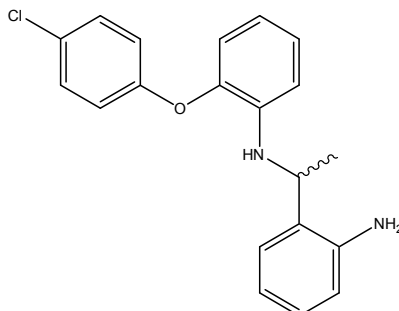
C<sub>20</sub>H<sub>17</sub>ClN<sub>2</sub>O<sub>3</sub>, MW 368.81,



The above compound was synthesised using a modified method from Harris *et al.*<sup>113</sup> Palladium acetate (15 mg, 10 mol%), rac-BINAP (45 mg, 10 mol%) and 1-(2-nitro-phenyl)-ethylamine hydrochloride (151 mg, 0.75 mmol) were placed into an over dried flask, this was evacuated and back filled with N<sub>2</sub>. To this was then added (via syringe) 1-bromo-2-phenoxy-4'chlorobenzene (190 mg, 0.68 mmol) and toluene (2 mL). This was stirred for 10 min at r.t. Sodium *t*-butoxide (195 mg, 2.04 mmol) and a further portion of toluene (2 mL) was then added. The resulting solution was heated to reflux for 24 h.<sup>113</sup> The slurry was then filtered through Celite and purified by flash chromatography (0-100 % DCM in hexane) to yield the desired product as a yellow oil, 120 mg, 48 % yield. R.f. 0.45 (1:1, Hexane: DCM), LCMS: *t*<sub>r</sub> = 3.85 min (90 % MeOH in water), *m/z* M-H 367.50, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 1.51 (3H, d, *J* = 6.8 Hz, CH<sub>3</sub>), 5.16 (1H, q, *J* = 6.4 Hz, CH), 6.28 (1H, dd, *J* = 1.2, 7.6 Hz, ArH), 6.56 (1H, td, *J* = 1.2, 7.6 Hz, ArH), 6.76 (1H, dd, *J* = 1.6, 8.4 Hz, ArH), 6.82 (1H, td, *J* = 1.2, 7.2 Hz, ArH), 6.89-6.93 (2H, m, ArH), 7.24-7.28 (2H, m, ArH), 7.31-7.35 (1H, m, ArH), 7.47 (1H, td, *J* = 1.2, 7.6 Hz, ArH), 7.54 (1H, dd, *J* = 1.2, 8.0 Hz, ArH), 7.88 (1H, dd, *J* = 1.2, 8.4 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 24.1 (CH<sub>3</sub>), 48.7 (CH), 112.3, 117.5, 118.7, 119.1, 124.7, 125.2, 127.3, 127.8 (ArCH), 127.9 (ArC), 129.7, 133.6 (ArCH), 138.4, 140.4, 142.6, 148.7, 156.1 (ArC).

**[2-(4-Chloro-phenoxy)-phenyl]-[1-(2-amino-phenyl)-ethyl]-amine, 183,**

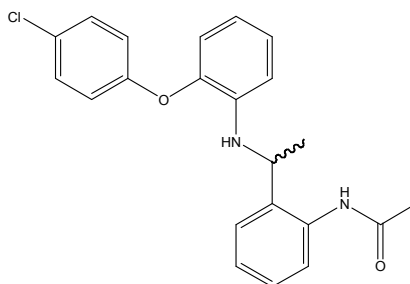
C<sub>20</sub>H<sub>19</sub>ClN<sub>2</sub>O, MW 338.83,



Using the general procedure for the reduction of the substituted 2-nitrobenzylalcohol, but with a shortened reaction time of 10min at reflux, the product was isolated as a yellow oil, 12 mg, 25 % yield. R.f. 0.32 (DCM), LCMS: *t*<sub>r</sub> = 2.81 min (90 % MeOH in

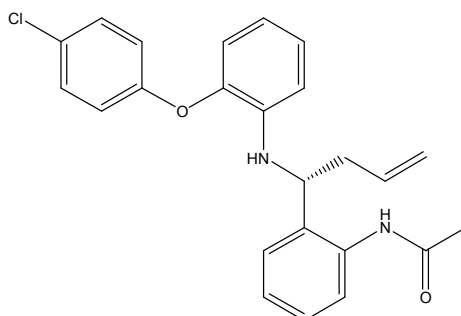
water),  $m/z$  M-H 337.60,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.53 (3H, d,  $J$  = 6.8 Hz,  $\text{CH}_3$ ), 4.06 (2H, br.s,  $\text{NH}_2$ ), 4.21 (1H, br.s, NH), 4.54-4.56 (1H, m, CH), 6.63-6.67 (2H, m, ArH), 6.70-6.77 (2H, m, ArH), 6.81 (1H, dd,  $J$  = 0.8, 7.6 Hz, ArH), 6.87-6.91 (2H, m, ArH), 6.95-6.99 (1H, m, ArH), 7.07 (1H, td,  $J$  = 0.8, 7.2 Hz, ArH), 7.19 (1H, dd,  $J$  = 1.2, 7.6 Hz, ArH), 7.23-7.27 (2H, m, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz): 20.2 ( $\text{CH}_3$ ), 50.1 (CH), 113.3, 116.6, 117.7, 118.6, 118.7, 119.3, 125.3, 126.7 (ArCH), 127.5, 127.7 (ArC), 128.0, 129.6 (ArCH), 139.5, 143.1, 144.8, 156.2 (ArC).

***N*-(2-(1-[2-(4-chloro-phenoxy)-phenylamino]-ethyl)-phenyl)-acetamide, 175**,  
 $\text{C}_{22}\text{H}_{21}\text{ClN}_2\text{O}_2$ , MW 380.87,



To a solution of [2-(4-chloro-phenoxy)-phenyl]-[1-(2-amino-phenyl)-ethyl]-amine (20 mg, 0.06 mmol) and TEA (0.008 mL) in DCM (1 mL) at 0 °C, was added acetyl chloride (0.009 mL) and the resulting solution was allowed to warm to r.t. and stirred for 1 h.  $\text{NaHCO}_3$  was then added and the mixture was repeatedly extracted with DCM, the organic layers were then washed with 1M HCl. The organic layers were combined, dried ( $\text{MgSO}_4$ ) and evaporated *in vacuo*. Purification by flash chromatography (0-10 % MeOH in DCM) afforded the desired product as an off white oil, 22 mg, 96 % yield. Analysis as previous (**182**).

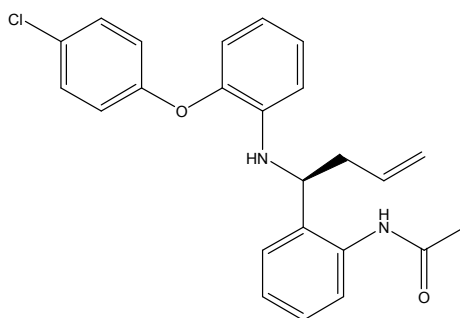
Chiral Separation of *R*-(-)-*N*-(2-(1-[2-(4-chloro-phenoxy)-phenylamino]-but-2-enyl)-phenyl)-acetamide, **184**,  $\text{C}_{24}\text{H}_{23}\text{ClN}_2\text{O}_2$ , MW 406.90,



m.p. 139-140 °C (from hexane/DCM), LCMS (Chiracel AD-H column):  $t_r$  = 11.5 min (80 % MeOH in water),  $m/z$  M-H 405.2, HPLC (Chiracel AD-H column):  $t_r$  = 9.00min (80 % MeOH in water), >99%,  $[\alpha]_D = -155.7$ ,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.86 (3H, s,  $\text{CH}_3$ ), 2.54-2.62 (2H, m,  $\text{CH}_2$ ), 4.31 (1H, t,  $J$  = 6.4 Hz,  $\text{CHNH}$ ), 4.60 (1H, s, NH), 5.10 (1H, s,  $\frac{1}{2}\text{CH}_2\text{CH}$ ), 5.13 (1H, d,  $J$  = 5.2 Hz,  $\frac{1}{2}\text{CH}_2\text{CH}$ ), 5.65-5.75 (1H, m,  $\text{CHCH}_2$ ),

6.66 (1H, d,  $J = 8.0$  Hz, ArH), 6.77 (1H, t,  $J = 8.0$  Hz, ArH), 6.87-6.96 (4H, m, ArH), 7.13 (1H, t,  $J = 7.6$  Hz, ArH), 7.26-7.31 (4H, m, ArH), 8.05 (1H, d,  $J = 8.4$  Hz, ArH), 9.40 (1H, br.s, NHCO).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz): 24.3 ( $\text{CH}_3$ ), 40.6 ( $\text{CH}_2$ ), 58.9 ( $\text{CH}_2$ ), 114.9, 118.0 (ArCH), 119.3 ( $\text{CH}_2$ ), 119.8, 120.0, 123.1, 124.7, 125.6 (ArCH), 128.0 (ArC), 128.2, 128.2, 129.8 (ArCH), 130.7 (ArC), 134.0 (CH), 136.9, 139.3, 143.5, 156.1 (ArC), 168.1 (CO). Anal. Calcd for  $\text{C}_{24}\text{H}_{23}\text{ClN}_2\text{O}_2 \cdot \frac{1}{2}\text{H}_2\text{O}$  C 69.31, H 5.82, N 6.74 %. Found: C 68.9, H 5.75, N 6.50 %. HRMS: Calcd for  $\text{C}_{24}\text{H}_{23}\text{ClN}_2\text{O}_2$  ( $\text{M}+\text{H}$ ) $^+$  407.1521, found ( $\text{M}+\text{H}$ ) $^+$  407.1503. X-Ray Crystallography used to determine absolute stereochemistry (See Appendix III).

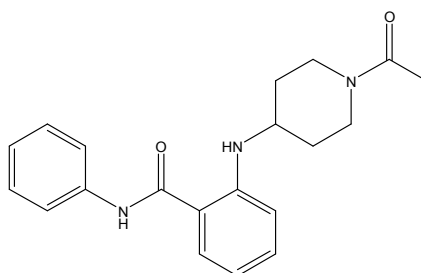
Chiral Separation of *S*-(+)- *N*-(2-(1-[2-(4-chloro-phenoxy)-phenylamino]-but-2-enyl)-phenyl)-acetamide, **185**,  $\text{C}_{24}\text{H}_{23}\text{ClN}_2\text{O}_2$ , MW 406.90,



m.p. 139-141 °C (from hexane/DCM), LCMS (Chiracel AD-H column):  $t_r = 14.8$  min (80 % MeOH in water),  $m/z$  M-H 405.4, HPLC (Chiracel AD-H column):  $t_r = 11.50$  min (90 % acetonitrile in water), >99 %,  $[\alpha]_D = +158.0$ ,  $^1\text{H}$  NMR: As **191**,  $^{13}\text{C}$  NMR: As **191**, Anal. Calcd for  $\text{C}_{24}\text{H}_{23}\text{ClN}_2\text{O}_2 \cdot \frac{1}{2}\text{H}_2\text{O}$  C 69.31, H 5.82, N 6.74 %. Found: C 69.7, H 5.74, N 6.75 %. HRMS: Calcd for  $\text{C}_{24}\text{H}_{23}\text{ClN}_2\text{O}_2$  ( $\text{M}+\text{H}$ ) $^+$  407.1521, found ( $\text{M}+\text{H}$ ) $^+$  407.1502.

### Experimental Details: Chapter 9: Synthesis of Compounds with an Amide Linked Hydrophobic Headgroup

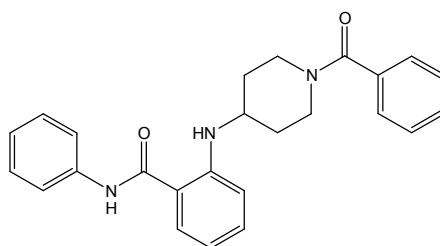
**2-(1-Acetyl-piperidin-4-ylamino)-*N*-phenyl-benzamide, 186**,  $\text{C}_{20}\text{H}_{23}\text{N}_3\text{O}_2$ , MW 337.42,



2-Aminobenzanilide (100 mg, 0.47 mmol), 1-acetyl-4-piperidone (0.12 mL, 0.96 mmol), sodium triacetoxyborohydride (0.25 g, 1.18 mmol) and AcOH (0.24 mL) were all mixed in toluene (2 mL) and subjected to microwave heating for 10 min and 120 °C.

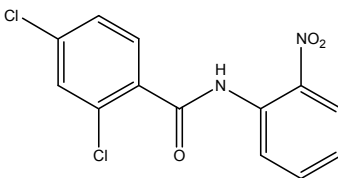
NaHCO<sub>3</sub> was added and the reaction was extracted with EtOAc, dried (MgSO<sub>4</sub>), and evaporated *in vacuo*. The crude product was purified by flash chromatography (0-10 MeOH in DCM) to afford the desired product as a cream solid, 75 mg, 47 % yield. R.f. 0.35 (5 % MeOH in DCM), LCMS  $t_r$  = 3.58 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min),  $m/z$  M+H 338.22, HPLC  $t_r$  = 6.72 min (70 % acetonitrile in water at 0.3 mL/min), 97 %, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 270 MHz)  $\delta$  1.25-1.35 (2H, m, CH<sub>2</sub>), 2.09 (3H, s, CH<sub>3</sub>), 2.40-2.47 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 2.77-2.86 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 3.19-3.23 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>) 3.50-3.60 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 3.70-3.91 (2H, m, CH<sub>2</sub>), 4.05-4.11 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 4.45 (1H, br.s, NH), 6.56-6.59 (2H, m, ArH), 7.06-7.11 (1H, m, ArH), 7.29-7.34 (2H, m, ArH), 7.59-7.63 (2H, m, ArH), 7.69-7.73 (2H, m, ArH), 7.88 (1H, s, NH). <sup>13</sup>CNMR (CDCl<sub>3</sub>, 68 MHz)  $\delta$  21.6 (CH<sub>3</sub>), 31.9, 32.7, 40.4, 45.1 (CH<sub>2</sub>), 48.7 (CH), 112.3, 120.2 (ArCH), 124.0 (ArC), 124.1, 129.1, 129.1 (ArCH), 138.5, 149.7 (ArC), 165.5, 169.1 (CO). HRMS: Calcd for C<sub>20</sub>H<sub>23</sub>N<sub>3</sub>O<sub>2</sub> (M+H)<sup>+</sup> 400.2020, found (M+Na)<sup>+</sup> 400.2017.

**2-(1-Benzoyl-piperidin-4-ylamino)-N-phenyl-benzamide, 187**, C<sub>25</sub>H<sub>25</sub>N<sub>3</sub>O<sub>2</sub>, MW 399.48,



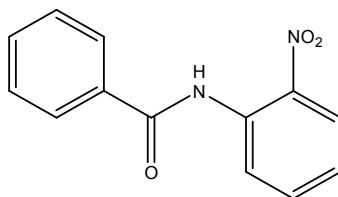
4-Aminobenzanilide (100 mg, 0.47 mmol), *N*-benzoyl-4-piperidone (190 mg, 0.96 mmol), sodium triacetoxyborohydride (0.25 g, 1.18 mmol) and AcOH (0.24 mL) were all mixed in toluene (2 mL) and subjected to microwave heating for 15 min at 100 °C. NaHCO<sub>3</sub> was added and the reaction was extracted with EtOAc, dried (MgSO<sub>4</sub>), and evaporated *in vacuo*. The crude product was purified by flash chromatography (0-100% EtOAc in hexane) to afford the desired product as a cream solid, 40 mg, 21 % yield. R.f. 0.65 (EtOAc), LCMS  $t_r$  = 4.19 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min),  $m/z$  M+H 400.50, HPLC  $t_r$  = 4.69 min (100 % MeOH at 0.4 mL/min), 99 %, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 270 MHz)  $\delta$  1.41-1.50 (2H, m, CH<sub>2</sub>), 2.04-2.19 (2H, m, CH<sub>2</sub>), 3.14 (2H, s, CH<sub>2</sub>), 3.63 (1H, s, CH<sub>2</sub>), 3.80 (1H, s, CH<sub>2</sub>), 4.00-4.05 (1H, m, CH<sub>2</sub>), 4.64 (1H, br.s. NH), 6.60-6.63 (2H, m, ArH), 7.34 (1H, t,  $J$  = 8.0 Hz, ArH), 7.39-7.47 (5H, m, ArH), 7.60 (2H, d,  $J$  = 7.6 Hz, ArH), 7.68 (1H, br.s, NH), 7.70-7.74 (2H, m, ArH). <sup>13</sup>CNMR (CDCl<sub>3</sub>, 101 MHz)  $\delta$  31.8 (CH<sub>3</sub>), 32.6, 40.1, 46.4 (CH<sub>2</sub>), 49.6 (CH), 112.1, 119.9 (ArCH), 123.9, 126.7, 128.5, 128.7, 129.0, 129.8 (ArCH), 135.5, 138.2, 149.4 (ArC), 165.0, 170.4 (CO). HRMS: Calcd for C<sub>25</sub>H<sub>25</sub>N<sub>3</sub>O<sub>2</sub> (M+H)<sup>+</sup> 400.2020, found (M+Na)<sup>+</sup> 400.2017.

**2,4-Dichloro-*N*-(3-nitro-phenyl)-benzamide, 191**, C<sub>13</sub>H<sub>8</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>, MW 311.12,



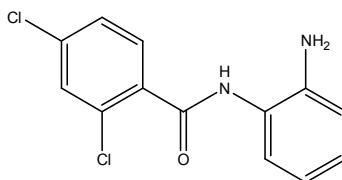
To a stirred solution of 2-nitroaniline (200 mg, 1.45 mmol) and K<sub>2</sub>CO<sub>3</sub> (600 mg, 4.35 mmol) in DCM (10 mL) was added 2,4-dichlorobenzoyl chloride (0.41 mL, 1.9 mmol). This was stirred at reflux for 5 h. DMAP (cat.) was added and the solution was stirred at reflux for a further 2 days. The reaction was quenched with NaHCO<sub>3</sub> and extracted with DCM and the organic layers were washed with HCl (1 M) and brine. These were dried (MgSO<sub>4</sub>) and evaporated *in vacuo* to afford the desired product as an off white solid, 330 mg, 73 % yield. R.f. 0.55 (DCM), m.p. 138-140 °C (from hexane), LCMS *t*<sub>r</sub>= 4.81 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min), *m/z* M+H 309.32, HPLC *t*<sub>r</sub>= 5.31 min (90 % acetonitrile in water at 0.5 mL/min), 89 %, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 270 MHz) δ 7.23-7.30 (3H, m, ArH), 7.66-7.76 (2H, m, ArH), 8.26 (1H, dd, *J* = 1.76, 8.67 Hz, ArH), 8.91 (1H, dd, *J* = 1.24, 8.67, ArH), 10.89 (1H, s, NH).

***N*-(3-Nitro-phenyl)-benzamide, 192**, C<sub>13</sub>H<sub>10</sub>N<sub>2</sub>O<sub>3</sub>, MW 242.23,



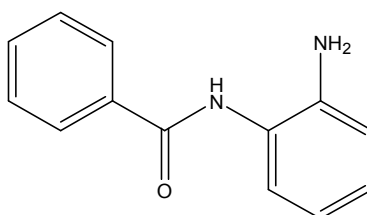
To a stirred solution of 2-nitroaniline (0.2 g, 1.45 mmol) and TEA (0.35 mL) in DCM (10 mL) at 0°C, was added benzoyl chloride (0.34 mL, 2.9 mmol). This was allowed to warm to r.t. and was stirred for 5 h. DMAP (cat.) was added and the solution was stirred at reflux for 18 h. The reaction was quenched with NaHCO<sub>3</sub> and extracted with DCM. The organic layers were washed with HCl (1 M) and brine. They were then dried (MgSO<sub>4</sub>) and evaporated *in-vacuo*. The crude product was purified by flash chromatography (0-100% DCM in hexane) to afford the desired product, 348 mg, 99 % yield. R.f. 0.63 (DCM), m.p. 87-90 °C (lit. 95-96 °C),<sup>152</sup> LCMS *t*<sub>r</sub>= 7.48 min (50% to 95% MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min), *m/z* M+H 243.37, HPLC *t*<sub>r</sub>= 12.23 min (70 % acetonitrile in 30 % H<sub>2</sub>O at 0.4 mL/min), 77 %, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 270 MHz) δ 7.19-7.26 (1H, m, ArH), 7.49-7.66 (3H, m, ArH and NH), 7.67-7.74 (1H, m, ArH), 7.97-8.01 (2H, m, ArH), 8.14-8.17 (1H, m, ArH), 8.28 (1H, dd, *J*=1.5, 8.4 Hz, ArH), 9.01 (1H, dd, *J*=1.2, 8.4 Hz, ArH).

***N*-(2-Amino-phenyl)-2,4-dichloro-benzamide, 193**, C<sub>13</sub>H<sub>10</sub>Cl<sub>2</sub>N<sub>2</sub>O, MW 281.14,



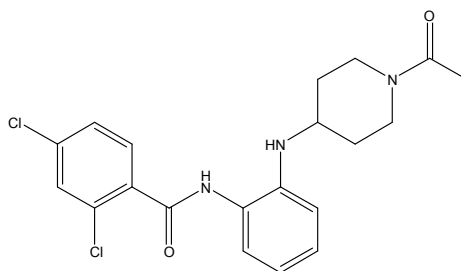
To a stirred mixture of iron powder (150 mg, 2.64 mmol) and NH<sub>4</sub>Cl (18 mg, 0.33 mmol) in EtOH (5 mL) and water (0.5 mL) at reflux, was added 2,4-dichloro-*N*-(3-nitro-phenyl)-benzamide (150 mg, 0.48 mmol). The mixture was stirred at reflux for 1 h and then allowed to cool and the solvent was removed *in vacuo*. The residue was re-dissolved in DCM (40 mL) and washed with sat. NaHCO<sub>3</sub> (40 mL). The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo* to afford the desired product as an off white solid, 118 mg, 83 % yield. R.f. 0.38 (DCM), m.p. 177-179 °C, LCMS *t*<sub>r</sub> = 1.04 min (95 % MeOH in water at 1.0 mL/min), *m/z* M+H 282.35, HPLC *t*<sub>r</sub> = 3.99 min (90 % acetonitrile in water at 0.5 mL/min), 98 %, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 270 MHz) δ 3.87 (2H, s, NH<sub>2</sub>), 6.82-6.89 (2H, m, ArH), 7.08-7.14 (1H, m, ArH), 7.35-7.40 (2H, m, ArH), 7.48 (1H, d, *J* = 1.97 Hz, ArH), 7.78 (1H, d, *J* = 8.4 Hz, ArH), 7.82 (1H, s, NH).

***N*-(2-Amino-phenyl)-benzamide, 194**, C<sub>13</sub>H<sub>12</sub>N<sub>2</sub>O, MW 212.25,



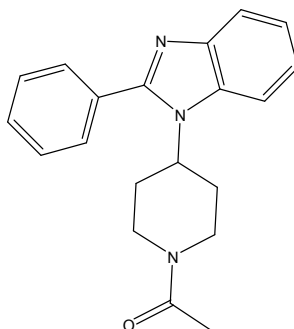
To a stirred mixture of iron powder (0.19 g, 3.41 mmol) and NH<sub>4</sub>Cl (0.023 g, 0.43 mmol) in EtOH (5 mL) and water (0.5 mL) at reflux, was added *N*-(3-nitro-phenyl)-benzamide (150 mg, 0.62 mmol). The mixture was stirred at reflux for 1.5 h allowed to cool and the solvent was removed *in vacuo*. The residue was re-dissolved in DCM (40 mL) and washed with sat. NaHCO<sub>3</sub> (40 mL). The organic layers were combined, dried (MgSO<sub>4</sub>), filtered and evaporated *in vacuo* to afford the desired product as a yellow waxy solid, 98 mg, 75 % yield. R.f. 0.33 (DCM), LCMS *t*<sub>r</sub> = 3.05 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min), *m/z* M+H 213.23, HPLC *t*<sub>r</sub> = 6.56 min (70 % acetonitrile in water at 0.5 mL/min), 97 %, <sup>1</sup>HNMR (CDCl<sub>3</sub>, 270 MHz) δ 3.87 (2H, s, NH<sub>2</sub>), 6.82-6.87 (2H, m, ArH), 7.07-7.13 (1H, m, ArH), 7.31-7.34 (1H, m, ArH), 7.42-7.59 (3H, m, ArH), 7.82 (1H, s, NH), 7.90 (2H, d, *J* = 6.9 Hz, ArH). This compound was previously synthesised using a different method, by Kettlera *et al.*<sup>117</sup>

***N*-[2-(1-Acetyl-piperidin-4-ylamino)-phenyl]-2,4-dichloro-benzamide, 195,**  
 $C_{20}H_{21}Cl_2N_3O_2$ , MW 406.31,



To a solution of *N*-(2-amino-phenyl)-2, 4-dichloro-benzamide (100 mg, 0.36 mmol) and 1-acetyl-4-piperidone (0.1 mL, 0.72 mmol) in toluene (2 mL), was added sodium triacetoxyborohydride (190 mg, 0.9 mmol) and AcOH (0.08 mL, 1.08 mmol). The solution was heated using a CEM microwave at 140 °C for 10 min.<sup>85</sup>  $NaHCO_3$  was added and the mixture was repeatedly extracted with EtOAc. The organic layers were combined and dried ( $MgSO_4$ ). The crude mixture was purified using flash chromatography (0-10 % MeOH in DCM) to afford an off-white oil, 61mg, 42 % yield. Rf: 0.3 (EtOAc), m.p. 107-109 °C, LCMS  $t_r$  = 4.04 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min),  $m/z$  M+H 407.42, HPLC  $t_r$  = 3.80 min (90 % acetonitrile in water at 1.0 mL/min), >99 %,  $^1H$ NMR ( $CDCl_3$ , 400 MHz) (a mixture of conformational isomers was obtained, the major isomer is reported here),  $\delta$  2.05 (3H, s,  $CH_3$ ), 2.14 (1H, d,  $J$  = 11.2Hz,  $\frac{1}{2}CH_2$ ), 2.29-2.36 (1H, m,  $\frac{1}{2}CH_2$ ), 2.41-2.52 (1H, m,  $\frac{1}{2}CH_2$ ), 2.87-2.94 (1H, m,  $\frac{1}{2}CH_2$ ), 3.15-3.22 (1H, m,  $\frac{1}{2}CH_2$ ), 3.48-3.54 (1H, m,  $\frac{1}{2}CH_2$ ), 3.72-3.77 (1H, m,  $\frac{1}{2}CH_2$ ), 3.86-4.11 (2H, m, NH and CH), 4.30- 4.35 (1H, m,  $\frac{1}{2}CH_2$ ), 6.78-6.81 (1H, m, ArH), 7.14-7.18 (1H, m, ArH), 7.27-7.31 (1H, m, ArH), 7.34 (1H, dd,  $J$  = 2.0, 8.0Hz, ArH), 7.39-7.42 (1H, m, ArH), 7.45 (1H, d,  $J$  = 2.0Hz, ArH), 7.67 (1H, d,  $J$  = 8.4Hz, ArH), 8.12 (1H, br.s, NHCO).  $^{13}C$ NMR ( $CDCl_3$ , 101 MHz) (a mixture of conformational isomers was obtained, the major isomer is reported here),  $\delta$  21.4 ( $CH_3$ ), 31.9, 32.5, 40.0, 44.8 ( $CH_2$ ), 49.9 (CH), 111.9, 113.9, 118.5 (ArCH), 120.1, 122.5, 123.9 (ArC), 125.9, 127.7, 130.0, 131.4 (ArCH), 137.1, 140.8 (ArC), 168.9, 168.8 (CO). HRMS: Calcd for  $C_{20}H_{21}Cl_2N_3O_2$  (M-H)<sup>+</sup> 405.1084, found (M-H)<sup>+</sup> 405.1085.

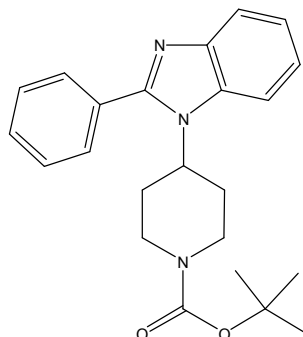
**1-[4-(2-Phenyl-benzoimidazol-1-yl)-piperidin-1-yl]-ethanone, 196,**  $C_{20}H_{21}N_3O$ , MW 319.40,





To a solution of *N*-(2-amino-phenyl)-benzamide (98 mg, 0.46 mmol), 1-acetyl-4-piperidone (0.114 mL, 0.92 mmol) in toluene (2 mL) was added sodium triacetoxyborohydride (0.25g, 1.15 mmol) and AcOH (0.08 mL, 1.4 mmol). The solution was heated using a CEM microwave at 140 °C for 10 min and then stirred at r.t. for 2 days.<sup>85</sup> NaHCO<sub>3</sub> was added and the mixture was extracted with EtOAc and dried (MgSO<sub>4</sub>). The crude mixture was purified using flash chromatography (0-100 % EtOAc in hexane) to afford a white solid, 112 mg, 72 % yield. mp. 207-209 °C, Rf: 0.6 (DCM), LCMS *t*<sub>r</sub> = 3.63 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min), *m/z* M+H 320.53, HPLC *t*<sub>r</sub> = 3.39 min (90 % acetonitrile in water at 1.0 mL/min), 95 %. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 1.95-2.09 (2H, m, CH<sub>2</sub>), 2.18 (3H, m, CH<sub>3</sub>), 2.45-2.57 (3H, m, ½CH<sub>2</sub> and CH<sub>2</sub>), 3.06-3.14 (1H, m, ½CH<sub>2</sub>), 3.96-4.00 (1H, m, ½CH<sub>2</sub>), 4.53-4.62 (1H, m, CH), 4.87 (1H, d, *J* = 8.8 Hz, ½CH<sub>2</sub>), 7.26-7.29 (2H, m, ArH), 7.50-7.56 (4H, m, ArH), 7.60-7.63 (2H, m, ArH), 7.81-7.83 (1H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz) δ 21.5 (CH<sub>3</sub>), 30.1, 30.9, 41.1, 45.8 (CH<sub>2</sub>), 54.9 (CH), 112.1, 120.5, 122.4, 122.7, 128.9, 129.4, 129.9 (ArCH), 130.6, 133.5, 143.6, 153.6 (ArC), 168.9 (CO). HRMS: Calcd for C<sub>20</sub>H<sub>21</sub>N<sub>3</sub>O (M+H)<sup>+</sup> 320.1757, found (M+Na)<sup>+</sup> 320.1758.

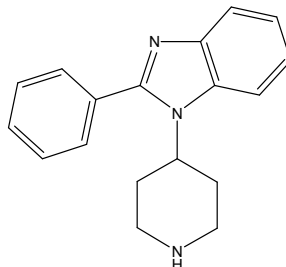
**4-(2-Phenyl-benzoimidazol-1-yl)-piperidine-1-carboxylic acid tert-butyl ester, 197,**  
C<sub>23</sub>H<sub>27</sub>N<sub>3</sub>O<sub>2</sub>, MW 377.48,



To a solution of *N*-(2-amino-phenyl)-benzamide (145 mg, 0.68 mmol) and 1-Boc-4-piperidine (272 mg, 1.4 mmol) in DCE (3 mL) was added NaBH(OAc)<sub>3</sub> (360 mg, 1.7 mmol) and AcOH (0.24 mL, 2 mmol). The resulting mixture was then subjected to microwave heating at 140 °C for 10 min. The reaction was quenched with sat. NaHCO<sub>3</sub>, extracted with DCM and dried (MgSO<sub>4</sub>).<sup>85</sup> The crude product was purified by flash chromatography (0-95 % EtOAc in hexane) to yield the desired product as a yellow oil, 147 mg, 57 % yield. R.f. 0.55 (EtOAc), LCMS: *t*<sub>r</sub> = 1.9 min (95 % MeOH in water), *m/z* M+H 375.15, HPLC: *t*<sub>r</sub> = 2.3 min (90 % acetonitrile in water), 98 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 1.51 (9H, s, (CH<sub>3</sub>)<sub>3</sub>), 1.89-1.92 (2H, m, CH<sub>2</sub>), 2.52-2.57 (2H, m, CH<sub>2</sub>), 2.73 (2H, br.s CH<sub>2</sub>), 3.82 (1H, br.s, ½CH<sub>2</sub>), 4.30 (1H, br.s, ½CH<sub>2</sub>), 4.46-4.54 (1H, m, CH), 7.24-7.31 (2H, m, ArH), 7.53-7.64 (6H, m, ArH), 7.82 (1H, dd, *J* = 2.4, 6.8Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): 28.4 ((CH<sub>3</sub>)<sub>3</sub>), 30.3, 34.1 (CH<sub>2</sub>), 55.1

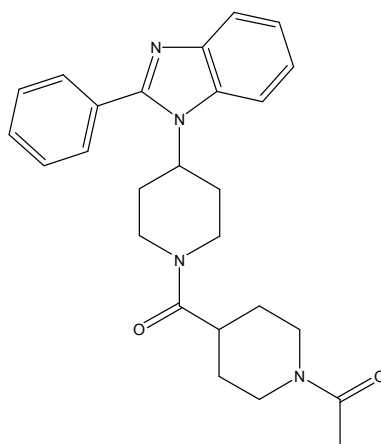
(CH), 67.7, 80.1 (CH<sub>2</sub>), 112.2, 120.4, 122.3, 122.5, 128.8, 129.4, 129.9 (ArCH), 130.7, 133.6, 143.6, 153.6 (ArC), 154.6 (CO). HRMS: Calcd for C<sub>23</sub>H<sub>27</sub>N<sub>3</sub>O<sub>2</sub> (M+H)<sup>+</sup> 378.2176, found (M+H)<sup>+</sup> 378.2189.

**2-Phenyl-1-piperidin-4-yl-1H-benzoimidazole, 198**, C<sub>18</sub>H<sub>19</sub>N<sub>3</sub>, MW 277.36,



A solution of 4-(2-phenyl-benzoimidazol-1-yl)-piperidine-1-carboxylic acid tert-butyl ester (130 mg, 0.35 mmol) in DCM (5 mL) was cooled to 0 °C and to this was added TFA (1 mL) and the resulting solution was stirred at r.t. for 30 min. The reaction solution was poured onto solid K<sub>2</sub>CO<sub>3</sub>, extracted with DCM, dried (MgSO<sub>4</sub>) and evaporated *in vacuo* to yield the desired product as an oil, 83 mg, 87 % yield. R.f. 0.1 (EtOAc), m.p. 185-188 °C (from hexane/DCM), LCMS: *t*<sub>r</sub> = 1.34 min (95 % MeOH in water), *m/z* M+H 278.09, HPLC: *t*<sub>r</sub> = 6.42 min (90 % acetonitrile in water), 87 %, (note: contained 10 % SM), <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.90 (2H, d, *J* = 12.4Hz, CH<sub>2</sub>), 2.55-2.70 (5H, m, 2CH<sub>2</sub>), 3.26 (2H, d, *J* = 9.9Hz, CH<sub>2</sub>), 4.40-4.46 (1H, m, CH), 7.22-7.28 (2H, m, ArH), 7.51-7.53 (3H, m, ArH), 7.59-7.62 (2H, m, ArH), 7.73-7.82 (2H, m, ArH).

**1-4-[4-(2-Phenyl-benzoimidazol-1-yl)-piperidine-1-carbonyl]-piperidin-1-yl-ethanone, 199**, C<sub>26</sub>H<sub>30</sub>N<sub>4</sub>O<sub>2</sub>, MW 430.54,

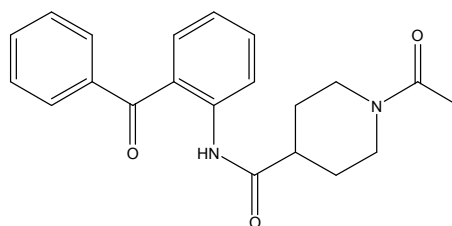


A solution of 2-phenyl-1-piperidin-4-yl-1H-benzoimidazole, (83 mg, 0.3 mmol) was dissolved in DCM (5 mL) and cooled to 0 °C, to this was added TEA (0.2 mL, 0.9 mmol) and 1-acetyl-4-carbonyl chloride (171 mg, 0.9 mmol). The resulting solution was stirred at r.t. for 4 h and was then quenched with sat. NaHCO<sub>3</sub>, extracted with DCM and dried (MgSO<sub>4</sub>). The crude material was then purified by flash chromatography (0-

10 % MeOH in EtOAc) to yield the desired product, 12 mg, 9 % yield. R.f. 0.35 (EtOAc with TEA), LCMS:  $t_r$  = 1.27 min (80 % MeOH in water),  $m/z$  M+H 431.15, HPLC:  $t_r$  = 1.86 min (70 % acetonitrile in water), 98 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.68-1.80 (4H, m,  $\text{CH}_2$ ), 1.99-2.07 (2H, m,  $\text{CH}_2$ ), 2.11 (3H, s,  $\text{CH}_3$ ), 2.49-2.68 (4H, m,  $\text{CH}_2$ ), 2.75-2.81 (1H, m, CH), 3.06-3.14 (2H, m,  $\text{CH}_2$ ), 3.24-3.39 (1H, m,  $\frac{1}{2}\text{CH}_2$ ), 3.87-3.94 (1H, m,  $\frac{1}{2}\text{CH}_2$ ), 4.08 (1H, d,  $J$  = 15.2 Hz,  $\frac{1}{2}\text{CH}_2$ ), 4.57-4.69 (1H, m, CH), 4.85-4.86 (1H, m,  $\frac{1}{2}\text{CH}_2$ ), 7.26-7.36 (2H, m, ArH), 7.47-7.51 (1H, m, ArH), 7.53-7.56 (3H, m, ArH), 7.60-7.63 (2H, m, ArH), 7.82-7.84 (1H, m, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz): 21.5 ( $\text{CH}_3$ ), 28.3, 28.6, 28.7, 29.0, 29.7, 30.3, 31.2, 34.5 ( $\text{CH}_2$ ), 38.3, 38.5 (CH), 40.3, 41.0, 41.6, 42.0, 44.9, 45.8, 45.9 ( $\text{CH}_2$ ), 55.0 (CH), 112.0, 120.6, 122.5, 122.7 (ArCH), 128.2, 128.7 (ArC), 129.0, 129.4, 130.1 (ArCH), 133.4, 143.6 (ArC), 172.6, 168.9 (CO). HRMS: Calcd for  $\text{C}_{26}\text{H}_{30}\text{N}_4\text{O}_2$  (M+H) $^+$  431.2442, found (M+H) $^+$  431.2422.

### Experimental Details: Chapter 10: Synthesis of Compounds with an Benzophenone Linked Hydrophobic Headgroup

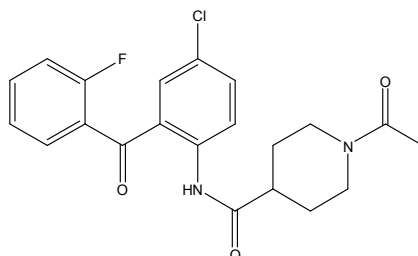
**1-Acetyl-piperidine-4-carboxylic acid (2-benzoyl-phenyl)-amide, 200**,  $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_3$ , MW 350.41,



The above compound was synthesised using a method reported by Kettler *et al.*<sup>117</sup> 1-Acetylpiperidine-4-carbonyl chloride (173 mg, 0.91 mmol) was dissolved in toluene (5 mL) and this was added to a solution of 2-aminobenzophenone (150 mg, 0.76 mmol) and pyridine (0.38 mL, 4.6 mmol) in toluene (15 mL). The resulting mixture was heated at reflux for 6 h. The toluene was removed *in-vacuo* and aqueous HCl solution (1 M) was added. The mixture was then extracted with DCM, dried ( $\text{MgSO}_4$ ) and evaporated to dryness. The crude product was purified by flash chromatography (0-10 % MeOH in DCM), to yield the desired product as a white solid, 129 mg, 48 % yield. R.f. 0.44 (10 % MeOH in EtOAc), m.p. 191-193 °C, LCMS:  $t_r$  = 0.94 min (95 % MeOH in water),  $m/z$  M-H 349.14, HPLC:  $t_r$  = 1.68 min (90 % acetonitrile in water), >99 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.67-1.87 (2H, m,  $\text{CH}_2$ ), 2.01-2.09 (2H, m,  $\text{CH}_2$ ), 2.09 (3H, s,  $\text{CH}_3$ ), 2.50-2.61 (1H, m, CH), 2.60-2.75 (1H, m,  $\frac{1}{2}\text{CH}_2$ ), 3.08-3.17 (1H, m,  $\frac{1}{2}\text{CH}_2$ ), 3.89 (1H, d,  $J$  = 13.9 Hz,  $\frac{1}{2}\text{CH}_2$ ), 4.64 (1H, d,  $J$  = 13.4 Hz,  $\frac{1}{2}\text{CH}_2$ ), 7.09 (1H, td,  $J$  = 0.97, 7.4 Hz, ArH), 7.46-7.51 (2H, m, ArH), 7.55-7.63 (3H, m, ArH), 7.67-7.69 (2H, m, ArH), 8.63-8.66 (1H, m, ArH), 11.10 (1H, s, NH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz): 21.6 ( $\text{CH}_3$ ), 28.5, 28.9, 41.1 ( $\text{CH}_2$ ), 44.7 (CH), 45.9 ( $\text{CH}_2$ ), 121.5, 122.4 (ArCH), 123.1

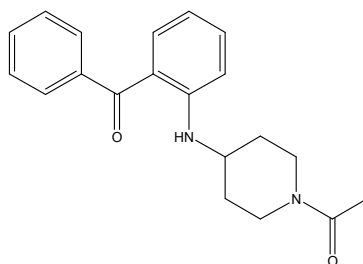
(ArC), 128.5, 129.9, 132.7, 134.0, 134.7 (ArCH), 138.7, 140.7 (ArC), 167.5, 169.0, 173.2 (CO). Anal. Calcd for  $C_{21}H_{22}N_2O_3$ : C 71.98, H 6.33, N 7.99 %. Found: C 71.6, H 6.29, N 8.00 %. HRMS: Calcd for  $C_{21}H_{22}N_2O_3$  ( $M+Na$ )<sup>+</sup> 373.1526, found ( $M+Na$ )<sup>+</sup> 373.1523.

**1-Acetyl-piperidine-4-carboxylic acid [4-chloro-2-(2-fluoro-benzoyl)-phenyl]-amide, 201**,  $C_{21}H_{20}ClFN_2O_3$ , MW 402.85,



The above compound was synthesised using a method reported by Kettler *et al.*<sup>117</sup> 1-Acetylpiperidine-4-carbonyl chloride (136 mg, 0.72 mmol) was dissolved in toluene (5 mL) and this was added to a solution of 2-amino-5-chloro-2'-fluorobenzophenone (150 mg, 0.6 mmol) in toluene (15 mL). The reaction was heated at reflux for 2 h. The toluene was removed *in vacuo* and 1 M HCl was added, the crude residue was then extracted with DCM, dried ( $MgSO_4$ ) and evaporated to dryness. The crude product was purified by flash chromatography (0-10 % MeOH in DCM) to yield the desired product as an off-white solid, 112 mg, 46 % yield. R.f. 0.35 (EtOAc), m.p. 202-204 °C, LCMS:  $t_r$  = 1.07 min (95 % MeOH in water),  $m/z$  M-H 401.10, HPLC:  $t_r$  = 1.79 min (90 % acetonitrile in water), >99 %,  $^1H$  NMR ( $CDCl_3$ , 270 MHz):  $\delta$  1.65-1.90 (2H, m,  $CH_2$ ), 2.02-2.08 (5H, m,  $CH_2$  and  $CH_3$ ), 2.51-2.75 (2H, m,  $\frac{1}{2}CH_2$  and CH), 3.08-3.18 (1H, m,  $\frac{1}{2}CH_2$ ), 3.89 (1H, d,  $J$  = 13.3 Hz,  $\frac{1}{2}CH_2$ ), 4.63 (1H, d,  $J$  = 13.3 Hz,  $\frac{1}{2}CH_2$ ), 7.15-7.31 (2H, m, ArH), 7.41-7.61 (4H, m, ArH), 8.71 (1H, d,  $J$  = 8.9 Hz, ArH), 11.35 (1H, br.s, NH).  $^{13}C$  NMR ( $CDCl_3$ , 68 MHz): 21.5 ( $CH_3$ ), 28.5, 28.9, 41.0 ( $CH_2$ ), 44.7 (CH), 45.8 ( $CH_2$ ), 116.6, 122.5 (ArCH), 123.7 (ArC), 130.3, 133.4, 133.8, 133.9, 135.4 (ArCH), 139.7, 157.7, 161.5 (ArC), 169.0, 173.3, 196.3 (CO). Anal. Calcd for  $C_{21}H_{20}ClFN_2O_3$ : C 62.61, H 5.00, N 6.95 %. Found: C 62.78, H 5.01, N 6.87 %. HRMS: Calcd for  $C_{21}H_{20}ClFN_2O_3$  ( $M+H$ )<sup>+</sup> 403.1219, found ( $M+H$ )<sup>+</sup> 403.1218.

**1-[4-(2-Benzoyl-phenylamino)-piperidin-1-yl]-ethanone, 202**,  $C_{20}H_{22}N_2O_2$ , MW 322.40,

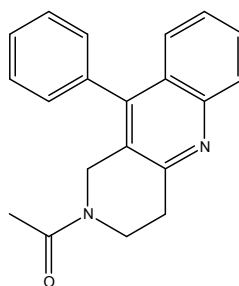


To a solution of 2-aminobenzophenone (197 mg, 1 mmol) and 1-acetyl-4-piperidine (282 mg, 2 mmol) in DCE (3 mL) was added  $\text{NaBH}(\text{OAc})_3$  (530 mg, 2.5 mmol) and AcOH (0.18 mL). The resulting solution was stirred at r.t. for 36 h.  $\text{NaHCO}_3$  was added and the mixture was extracted with DCM. The crude product was purified by flash chromatography (0-10 % MeOH in EtOAc) to yield the desired product as a yellow oil, 116 mg, 36 % yield. R.f. 0.58 (10 % MeOH in EtOAc), LCMS:  $t_r$  = 4.39 min (50 % to 95 % MeOH in water),  $m/z$  M+H 323.14, HPLC:  $t_r$  = 2.03 min (90 % acetonitrile in water), 95 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.48-1.68 (2H, m,  $\text{CH}_2$ ), 2.00-2.10 (5H, m,  $\text{CH}_3$  and  $\text{CH}_2$ ), 3.10-3.34 (2H, m,  $\text{CH}_2$ ), 3.67-3.82 (2H, m,  $\text{CH}_2$ ), 4.19-4.28 (1H, m,  $\text{CH}_2$ ), 6.50-6.56 (1H, m, ArH), 6.78 (1H, d,  $J$  = 8.4 Hz, ArH), 7.33-7.59 (7H, m, 6ArH and NH), 8.78 (1H, d,  $J$  = 7.2 Hz, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz): 21.6 ( $\text{CH}_3$ ), 31.4, 32.2, 39.8, 44.7 ( $\text{CH}_2$ ), 48.4 (CH), 111.8, 114.1, 128.2, 129.1, 130.9, 135.1, 136.0 (ArCH), 150.6, 169.0 (CO). HRMS: Calcd for  $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_2$  (M+H+Na) $^+$  345.1573, found (M+H+Na) $^+$  345.1565.

### General Procedure for the Friedländer Cyclisation

The desired benzophenone (1 mmol) and ketone (2 mmol) were dissolved in AcOH (2 mL). The resulting solution was subjected to microwave heating for 5 min at 160 °C: Saturated  $\text{NaHCO}_3$  was then added and the mixture was extracted with DCM, dried ( $\text{MgSO}_4$ ) and purified by flash chromatography to yield the desired quinoline product as detailed below.

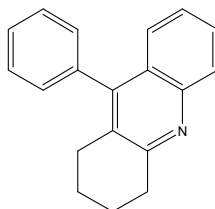
**1-(10-Phenyl-3,4-dihydro-1H-benzo[*b*][1,6]naphthyridin-2-yl)-ethanone, 203,**  
 $\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}$ , MW 302.37,



Following the general procedure for the Friedländer Cyclisation and flash chromatography it was found that the product was still contaminated with the 1-acetyl-4-piperidone starting material. This was removed by use of PS-TsNHNH<sub>2</sub>. The crude material was dissolved in DCM (~10 mL/g) and the resin was added (3 eq, 2.8 mmol/g), this was then stirred at r.t. for 1 h. The resin was removed by filtration and flash chromatography isolated the desired product as a white solid, 206 mg, 68 % yield. R.f. 0.33 (10 % MeOH in DCM), m.p. 163-165 °C (from hexane), (lit. ref. 166-167 °C<sup>153</sup>), LCMS:  $t_r$  = 0.95 min (95 % MeOH in water),  $m/z$  M+H 303.09, HPLC:  $t_r$  = 1.83 min (90 % acetonitrile in water), 99 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.95, 2.16 (3H,  $\text{CH}_3$ ),

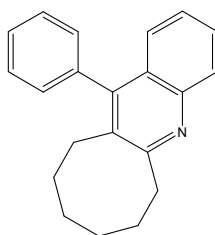
3.29-3.32 (2H, m, CH<sub>2</sub>), 3.83-3.99 (2H, m, CH<sub>2</sub>), 4.43, 4.60 (2H, CH<sub>2</sub>), 7.23-7.28 (2H, m, ArH), 7.38-7.39 (2H, m, ArH), 7.47-7.59 (3H, m, ArH), 7.62-7.70 (1H, m, ArH), 8.04 (1H, t, *J* = 6.6 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): 21.4, 21.9 (CH<sub>3</sub>), 32.8, 33.9, 39.8, 42.6, 43.8, 46.5 (CH<sub>2</sub>), 124.0, 124.6 (ArC), 125.9, 126.0, 126.2, 126.3 (ArCH), 126.4, 126.8 (ArC), 128.4, 128.5, 128.6, 128.7, 128.9, 129.0, 129.1, 129.3, 129.5 (ArCH), 135.1, 135.2, 145.1, 146.1, 146.6, 146.9, 155.3, 156.3 (ArC), 169.3, 169.4 (CO). Anal. Calcd for C<sub>20</sub>H<sub>18</sub>N<sub>2</sub>O (+ ½mole AcOH): C 75.9, H 6.1, N 8.4 %. Found: C 76.1, H 6.2, N 9.0 %. HRMS: Calcd for C<sub>20</sub>H<sub>18</sub>N<sub>2</sub>O (M+H)<sup>+</sup> 303.1492, found (M+H)<sup>+</sup> 303.1491. IR: 2800 (m), 1650 (s), 1300 (m), 1030 (m). X-Ray crystallography was used to identify structure, see Appendix III. This compound was previously synthesised, via a different method by Khaldeeva *et al.*<sup>153</sup>

**9-Phenyl-1,2,3,4-tetrahydro-acridine, 204**, C<sub>19</sub>H<sub>17</sub>N, MW 259.34,



Following the general procedure for the Friedländer Cyclisation the desired product was isolated, 246 mg, 94 % yield. R.f. 0.7 (10 % MeOH in DCM), m.p. 137-139 °C, (lit. 139-141 °C<sup>154</sup>), LCMS: *t<sub>r</sub>* = 4.19 min (50 % to 95 % MeOH in water at 0.5 mL/min to 1.0 mL/min over 5 min), *m/z* M+H 400.50, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.7-1.79 (2H, m, CH<sub>2</sub>), 1.90-2.01 (2H, m, CH<sub>2</sub>), 2.59 (2H, t, *J* = 6.5 Hz, CH<sub>2</sub>), 3.19 (2H, *J* = 6.4 Hz, CH<sub>2</sub>), 7.20-7.31 (4H, m, ArH), 7.4-7.65 (4H, m, ArH), 8.00 (1H, d, *J* = 8.2 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): 23.0, 23.1, 28.2, 34.4 (CH<sub>2</sub>), 125.5, 125.9 (ArCH), 127.0 (ArC), 127.8, 128.4, 128.7, 129.2 (ArCH), 137.2, 147.0, 159.2 (ArC). This compound has been previously synthesised using a different method, by Shaabani *et al.*<sup>154</sup>

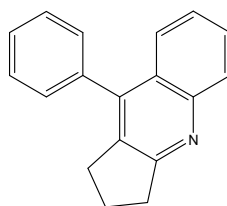
**12-Phenyl-6,7,8,9,10,11-hexahydro-cycloocta[b]quinoline, 205**, C<sub>21</sub>H<sub>21</sub>N, MW 287.40,<sup>155</sup>



Following the general procedure for the Friedländer Cyclisation the desired product was isolated as an off-white solid, 267 mg, 93 % yield. R.f. 0.3 (DCM), m.p. 120-122 °C, LCMS: *t<sub>r</sub>* = 1.78 min (95 % MeOH in water), *m/z* M+H 288.10, HPLC: *t<sub>r</sub>* = 4.9 min (90 % acetonitrile in water), 98 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz): δ 1.30-1.51 (6H, m, CH<sub>2</sub>),

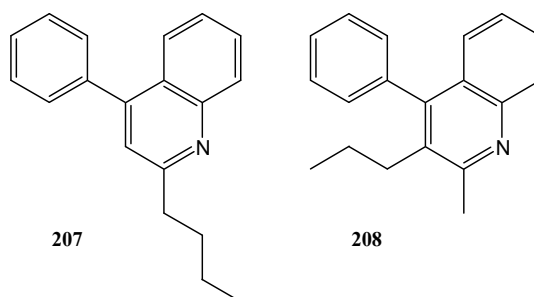
1.90-1.98 (2H, m, CH<sub>2</sub>), 2.76 (2H, t,  $J = 5.7$  Hz, CH<sub>2</sub>), 3.22 (3H, t,  $J = 6.2$  Hz, CH<sub>2</sub>), 7.18-7.32 (4H, m, ArH), 7.45-7.51 (3H, m, ArH), 7.55-7.52 (1H, m, ArH), 8.05 (1H, d,  $J = 7.9$  Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): 25.9, 26.8, 28.2, 31.2, 31.4, 36.5 (CH<sub>2</sub>), 125.5, 126.2 (ArCH), 127.3 (ArC), 127.7, 128.3, 128.4, 128.6, 129.4 (ArCH), 131.9, 137.7, 146.5, 163.6 (ArC). HRMS: Calcd for C<sub>21</sub>H<sub>21</sub>N (M+H)<sup>+</sup> 288.1747, found (M+H)<sup>+</sup> 288.1756. Anal. Calcd for C<sub>21</sub>H<sub>21</sub>N, C 87.76, H 7.36, N 4.87 %. Found: C 87.40, H 7.32, N 4.87 %. This compound has been previously synthesised using a different method, by Bose *et al.*<sup>155</sup>

**9-Phenyl-2,3-dihydro-1H-cyclopenta[b]quinoline, 206**, C<sub>18</sub>H<sub>15</sub>N, MW 245.32,



Following the general procedure for the Friedländer Cyclisation the desired product was isolated as an off-white solid, 243 mg, 99 % yield. R.f. 0.32 (DCM), m.p. 132-134 °C, (lit. 131-132 °C<sup>154</sup>), LCMS:  $t_r = 1.5$  min (95 % MeOH in water),  $m/z$  M+H 245.90, HPLC:  $t_r = 3.2$  min (90 % acetonitrile in water), >99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  2.10-2.20 (2H, m, CH<sub>2</sub>), 2.89 (2H, t,  $J = 7.4$  Hz, CH<sub>2</sub>), 3.23 (2H, t,  $J = 7.7$  Hz, CH<sub>2</sub>), 7.32-7.65 (8H, m, ArH), 8.06-8.08 (1H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): 23.6, 30.4, 35.2 (CH<sub>2</sub>), 126.6, 125.7 (ArCH), 126.3 (ArC), 128.1, 128.4, 128.6, 128.7, 129.4 (ArCH), 133.8, 136.8, 143.0, 147.9, 167.5 (ArC). HRMS: Calcd for C<sub>18</sub>H<sub>15</sub>N (M+H)<sup>+</sup> 246.1277, found (M+H)<sup>+</sup> 246.1250. Anal. Calcd for C<sub>18</sub>H<sub>15</sub>N, C 88.13, H 6.16, N 5.71 %. Found: C 87.90, H 6.13, N 5.76 %. This compound has been previously synthesised using a different method, by Shaabani *et al.*<sup>154</sup>

**2-Butyl-4-phenyl-quinoline, 207**, C<sub>19</sub>H<sub>19</sub>N, MW 261.36 and **2-methyl-4-phenyl-3-propyl-quinoline, 208**, C<sub>19</sub>H<sub>19</sub>N, MW 261.36,

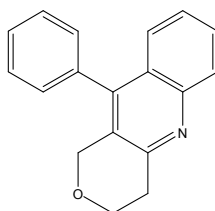


Following the general procedure for the Friedländer Cyclisation the desired products were synthesised and isolated. Overall yield 171 mg, 66 %, selectivity 1:1.9 (A:B).

**207: 2-Butyl-4-phenyl-quinoline** was isolated as an off-white oil, 59 mg, 23 % yield. R.f. 0.38 (DCM), LCMS:  $t_r$  = 1.54 min (95 % MeOH in water),  $m/z$  M+H 261.96, HPLC:  $t_r$  = 4.75 min (90 % acetonitrile in water), 93 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz): 0.96 (3H, t,  $J$  = 7.2 Hz,  $\text{CH}_3$ ), 1.39-1.52 (2H, m,  $\text{CH}_2$ ), 1.79-1.86 (2H, m,  $\text{CH}_2$ ), 2.99 (2H, t,  $J$  = 7.9 Hz,  $\text{CH}_2$ ), 7.39-7.59 (7H, m, ArH), 7.62-7.71 (1H, m, ArH), 8.08-8.11 (1H, m, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz): 15.0 ( $\text{CH}_3$ ), 22.9, 32.4, 39.3 ( $\text{CH}_2$ ), 121.7 (ArCH), 125.3 (ArC), 125.7, 125.8, 128.4, 128.6, 129.3, 129.3, 129.6 (ArCH), 138.4, 148.5, 148.6, 148.6, 167.8 (ArC). HRMS: Calcd for  $\text{C}_{19}\text{H}_{19}\text{N}$  (M+H) $^+$  262.1590, found (M+H) $^+$  262.1598. This compound has been previously synthesised via a different route by Kobayashi *et al.*<sup>156</sup>

**208: 2-Methyl-4-phenyl-3-propyl-quinoline** was isolated as a white solid, 112 mg, 43 % yield. R.f. 0.24 (DCM), m.p. = 114-116 °C, LCMS:  $t_r$  = 1.41 min (95 % MeOH in water),  $m/z$  M+H 261.96, HPLC:  $t_r$  = 3.87 min (90 % acetonitrile in water), >99 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  0.81 (3H, t,  $J$  = 7.4 Hz,  $\text{CH}_3\text{CH}_2$ ), 1.40-1.49 (2H, m,  $\text{CH}_2$ ), 2.48-2.54 (2H, m,  $\text{CH}_2$ ), 2.80 (3H, s,  $\text{CH}_3\text{Ar}$ ), 7.20-7.32 (3H, m, ArH), 7.44-7.51 (4H, m, ArH), 7.54-7.61 (1H, m, ArH), 8.03 (1H, dd,  $J$  = 0.5, 8.4 Hz, ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz): 14.5 ( $\text{CH}_3$ ), 23.6 ( $\text{CH}_2$ ), 23.9 ( $\text{CH}_3$ ), 32.5 ( $\text{CH}_3$ ), 125.4, 126.2 (ArCH), 127.1 (ArC), 127.6, 128.2, 128.3, 128.4, 129.3 (ArCH), 132.1, 137.4, 145.9, 146.5, 158.6 (ArC). Anal. Calcd for  $\text{C}_{19}\text{H}_{19}\text{N}$ : C 87.31, H 7.33, N 5.36 %. Found: C 86.9, H 7.48, N 5.26 %. HRMS: Calcd for  $\text{C}_{19}\text{H}_{19}\text{N}$  (M+H) $^+$  262.1590, found (M+H) $^+$  262.1591.

**10-Phenyl-3,4-dihydro-1H-pyrano[4,3-*b*]quinoline, 209**,  $\text{C}_{18}\text{H}_{15}\text{NO}$ , MW 261.32,

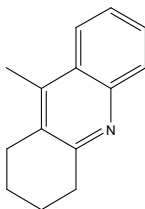


Following the general procedure for the Friedländer Cyclisation the desired product was isolated as a yellow solid, 260 mg, 99 % yield. R.f. 0.65 (10 % MeOH in DCM), m.p. = 146-148 °C, (lit. 130°C<sup>157</sup>), LCMS:  $t_r$  = 1.35 min (95 % MeOH in water),  $m/z$  M-H 262.09, HPLC:  $t_r$  = 2.41 min (90 % acetonitrile in water), >99 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 270 MHz):  $\delta$  1.93 (2H, s,  $\text{CH}_2$ ), 2.24 (2H, s,  $\text{CH}_2$ ), 4.80 (2H, s,  $\text{CH}_2$ ), 7.02-7.12 (2H, m, ArH), 7.21-7.30 (4H, m, ArH), 8.20 (1H, dd,  $J$  = 7.7 Hz, ArH), 9.89 (1H, s, NHCO).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz): 22.1, 24.6 ( $\text{CH}_2$ ), 49.8 ( $\text{CH}_2$ ), 118.1, 120.7, 122.1, 123.0, 124.0 (ArCH), 125.0 (ArC), 129.2 (ArCH), 129.8 (ArC), 130.1, 130.2, 131.5 (ArCH), 137.7, 153.2, 153.8 (ArC), 169.5, 172.7 (CO). HRMS: Calcd for  $\text{C}_{18}\text{H}_{15}\text{NO}_3$  (M+H) $^+$  262.1226, found (M+H) $^+$  262.1223. Anal. Calcd for  $\text{C}_{18}\text{H}_{15}\text{NO}$ , C 82.73, H 5.79, N



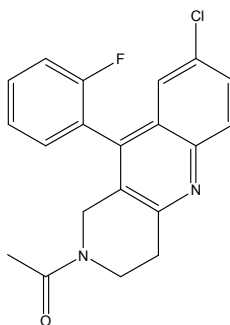
5.36 %. Found: C 82.30, H 5.74, N 5.38 %. This compound was previously synthesised, using a different route, by Kempter *et al.*<sup>157</sup>

**9-Methyl-acridine, 210**, C<sub>14</sub>H<sub>11</sub>N, MW 193.24,



Following the general procedure for the Friedländer Cyclisation the desired product was isolated as a yellow oil, 180 mg, 91 % yield. R.f. 0.14 (DCM), LCMS:  $t_r$  = 1.32 min (95 % MeOH in water),  $m/z$  M+H 197.71, HPLC:  $t_r$  = 4.8 min (90 % acetonitrile in water), >99 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  1.88-1.92 (4H, m, 2CH<sub>2</sub>), 2.52 (3H, s, CH<sub>3</sub>), 2.86 (2H, br.s, CH<sub>2</sub>), 3.10-3.12 (2H, m, CH<sub>2</sub>), 7.41-7.47 (1H, m, ArH), 7.59 (1H, td,  $J$  = 1.4, 6.9 Hz, ArH), 7.93 (1H, dd,  $J$  = 0.81, 8.5 Hz, ArH), 8.00 (1H, d,  $J$  = 8.5 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): 13.8 (CH<sub>3</sub>), 22.7, 23.2, 27.2, 33.9 (CH<sub>2</sub>), 123.4, 125.5 (ArCH), 126.9 (ArC), 128.3, 128.5 (ArCH), 128.6, 128.9, 142.1, 145.3, 158.5, 171.0 (ArC). HRMS: Calcd for C<sub>14</sub>H<sub>11</sub>N (M+H)<sup>+</sup> 198.1277, found (M+H)<sup>+</sup> 198.1271. This compound was previously synthesised, using a different route, by Wang *et al.*<sup>158</sup>

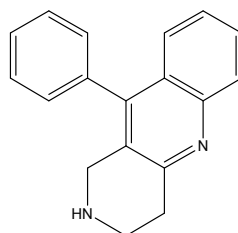
**1-[8-Chloro-10-(2-fluoro-phenyl)-3,4-dihydro-1H-benzo[*b*][1,6] naphthyridin-2-yl]-ethanone, 211**, C<sub>20</sub>H<sub>16</sub>ClFN<sub>2</sub>O, MW 354.81,



Following the general procedure for the Friedländer Cyclisation and flash chromatography it was found that the product was still contaminated with 1-acetyl-4-piperidone. This was removed by use of PS-TsNHNH<sub>2</sub>, the crude material was dissolved in DCM (~10mL/g) and the resin was added (3eq, 2.8mmol/g), this was stirred at r.t. for 1h. The resin was removed by filtration and flash chromatography isolated the desired product as a white solid, 248 mg, 70 % yield. R.f. 0.30 (EtOAc), m.p. 196-199 °C, LCMS:  $t_r$  = 1.03 min (95 % MeOH in water),  $m/z$  M+H 355.19, HPLC:  $t_r$  = 2.07 min (90 % acetonitrile in water), 98 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  2.18 (3H, s, CH<sub>3</sub>), 3.27-3.33 (2H, m, CH<sub>2</sub>), 3.81-4.00 (2H, m, CH<sub>2</sub>), 4.37-4.45 (1H, m,  $\frac{1}{2}$ CH<sub>2</sub>), 4.75, 4.82 (1H,  $\frac{1}{2}$ CH<sub>2</sub>), 7.19-7.39 (4H, m, ArH), 7.50-7.64 (2H, m, ArH), 7.96-8.02 (1H, m, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): 21.7, 21.8 (CH<sub>3</sub>), 32.9, 34.0, 39.7,

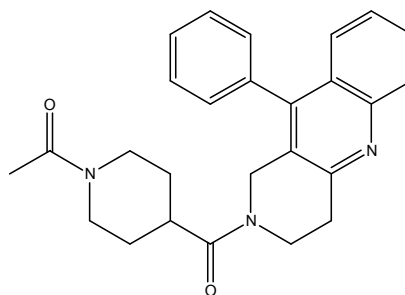
42.4, 43.7, 46.4 (CH<sub>2</sub>), 116.7 (d,  $J=21.8$  Hz, ArCH), 122.0 (ArC), 124.3 (ArCH), 125.1 (d,  $J=3.7$  Hz, ArCH), 126.8 (ArC), 130.5, 130.6, 131.0, 131.5 (ArCH), 132.5, 139.0, 155.7, 157.6, 161.3 (ArC), 169.2, 169.4 (CO). Anal. Calcd for C<sub>20</sub>H<sub>16</sub>ClFN<sub>2</sub>O: C 67.70, H 4.55, N 7.90%. Found: C 67.8, H 4.57, N 7.84%. HRMS: Calcd for C<sub>20</sub>H<sub>16</sub>ClFN<sub>2</sub>O (M+Na)<sup>+</sup> 377.0817, found (M+Na)<sup>+</sup> 377.0827.

**10-Phenyl-1,2,3,4-tetrahydro-benzo[*b*][1,6]naphthyridine, 212**, C<sub>18</sub>H<sub>16</sub>N<sub>2</sub>, MW 260.33,



Following the general procedure for the Friedländer Cyclisation using 2-aminobenzophenone (197 mg) and 1-Boc-4-piperidone (300 mg) and subsequent purification (0-10 % MeOH in EtOAc, with 5 % TEA) the desired product was isolated as a yellow oil, 170 mg, 65 % yield. R.f. 0.12 (10 % MeOH in EtOAc), LCMS:  $t_r$  = 1.70 min (95 % MeOH in water),  $m/z$  M+H 261.08, HPLC:  $t_r$  = 3.20 min (90 % acetonitrile in water), 96 %, <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz):  $\delta$  2.05 (1H, br.s, NH), 3.21-3.30 (4H, m, 2CH<sub>2</sub>), 3.84 (2H, s, CH<sub>2</sub>), 7.20-7.25 (3H, m, ArH), 7.31-7.33 (2H, m, ArH), 7.45-7.53 (2H, m, ArH), 7.57-7.63 (1H, m, ArH), 8.01 (1H, dd,  $J$  = 0.8, 9.1 Hz, ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz): 34.3, 44.1, 47.5 (CH<sub>2</sub>), 125.8, 125.9 (ArCH), 126.6, 127.0 (ArC), 128.2, 128.5, 128.8, 129.0 (ArCH), 136.0, 144.9, 146.8, 156.6 (ArC).

**1-[4-(10-Phenyl-3,4-dihydro-1*H*-benzo[*b*][1,6]naphthyridine-2-carbonyl)-cyclohexyl]-ethanone, 213**, C<sub>27</sub>H<sub>28</sub>N<sub>2</sub>O<sub>2</sub>, MW 413.41,



A solution of 10-phenyl-1,2,3,4-tetrahydro-benzo[*b*][1,6]naphthyridine (195 mg, 0.38 mmol) in DCM (10 mL) was cooled in an ice bath and to this was added 1-acetyl-piperidine-4-carbonyl chloride (282 mg, 0.76 mmol) and TEA (0.46 mL). The resulting solution was stirred at r.t. for 2 days. NaHCO<sub>3</sub> was added and the mixture was extracted with DCM. The organic portions were washed with 1M HCl, dried (MgSO<sub>4</sub>) and purified using flash chromatography (0-10 % MeOH in EtOAc) to afford the title compound as a cream oil, 66 mg, 21 % yield. R.f. 0.65 (EtOAc), LCMS:  $t_r$  = 0.93 min

(95 % MeOH in water),  $m/z$  M+H 414.20, HPLC:  $t_r$  = 1.65 min (90 % acetonitrile in water), 99 %,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  (Multiple signals observed due to restricted rotation and therefore the presence of rotamers) 1.62-1.79 (3H, m,  $\text{CH}_2$  and  $\frac{1}{2}\text{CH}_2$ ), 1.98 (1.3H, s,  $\text{CH}_3$ ), 2.02 (1.7H, s,  $\text{CH}_3$ ), 2.25-2.32 (1H, m,  $\frac{1}{2}\text{CH}_2$ ), 2.66 (1H, t,  $J=12.0\text{Hz}$ ,  $\frac{1}{2}\text{CH}_2$ ), 2.75-2.84 (1H, m,  $\frac{1}{2}\text{CH}_2$ ), 3.04-3.11 (1H, m,  $\frac{1}{2}\text{CH}_2$ ), 3.23-3.31 (2H, m,  $\text{CH}_2$ ), 3.67-3.93 (3H, m,  $\text{CH}_2$  and CH), 4.38-4.63 (3H, m,  $\text{CH}_2$  and  $\frac{1}{2}\text{CH}_2$ ), 7.20 (2H, t,  $J=9.6\text{ Hz}$ , ArH), 7.30-7.40 (2H, m, ArH), 7.42-7.51 (3H, m, ArH), 7.60-7.65 (1H, m, ArH), 7.99 (1H, t,  $J=11.2\text{ Hz}$ , ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz): 21.4 ( $\text{CH}_3$ ), 28.1, 28.3, 28.4, 28.7, 32.5, 34.2 ( $\text{CH}_2$ ), 38.4, 39.0 (CH), 40.5, 40.8, 42.9, 43.1, 45.4, 45.5, 45.7, 45.8 ( $\text{CH}_2$ ), 124.2, 124.4 (ArC), 126.0, 126.3, 126.4, 126.7, 128.3, 128.5, 128.7, 128.8, 128.9, 129.0, 129.1, 129.4, 129.6 (ArCH), 135.0, 135.2, 144.7, 146.2, 147.1, 154.8, 156.3, 168.9 (ArC), 172.5, 173.0 (CO). HRMS: Calcd for  $\text{C}_{27}\text{H}_{28}\text{N}_2\text{O}_2$  (M+H) $^+$  414.2176, found (M+H) $^+$  414.2192.

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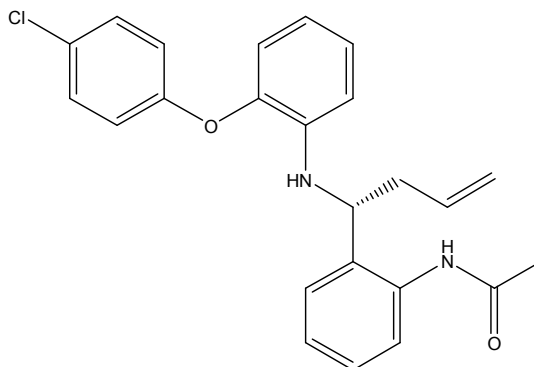


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## Appendix I: X-ray Crystallography Data

### X-ray crystallography data for 184



Identification Code	k07farm3
Empirical Formula	C <sub>24</sub> H <sub>23</sub> Cl N <sub>2</sub> O <sub>2</sub>
Formula Weight	406.89
Temperature	150(2) K
Wavelength	0.71073 Å
Crystal System	Orthorhombic
Space Group	P212121
Unit cell dimensions	a = 8.9500(0) Å $\alpha = 90^\circ$ b = 10.4450(0) Å $\beta = 90^\circ$ c = 22.6800(2) Å $\gamma = 90^\circ$
Crystal Size	0.35 x 0.25 x 0.25 mm

Notes:

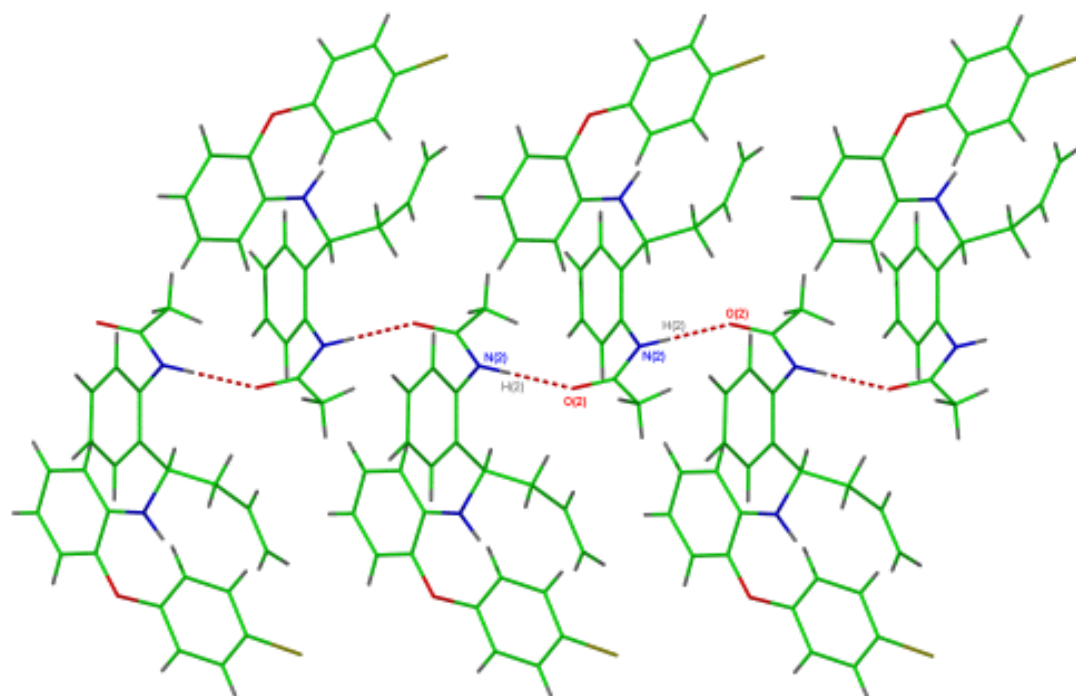
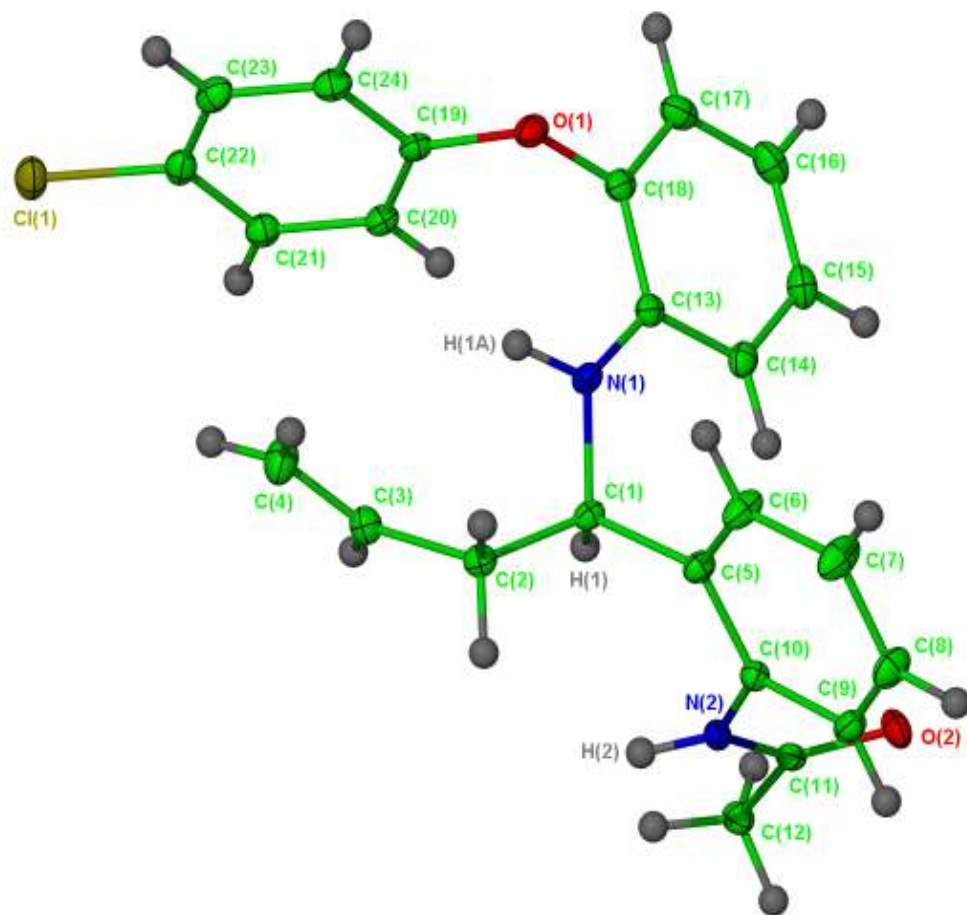
H1A and H2 (attached to N1 and N2, respectively) were located and refined at a distance of 0.9 Å from the parent nitrogen atoms. Hydrogen bonded polymers in the gross structure:

Hydrogen bonds with  $H \cdots A < r(A) + 2.000$  Angstroms and  $\angle DHA > 110$  deg.

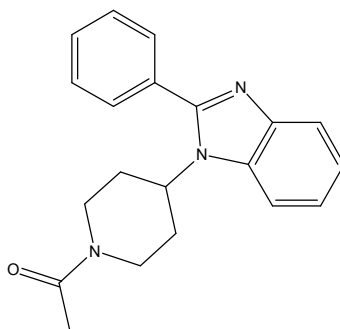
D-H	d(D-H)	d(H..A)	$\angle DHA$	d(D..A)	A
N1-H1A	0.906				
N2-H2	0.898	1.921	177.51	2.818	O2 [ x+1/2, -y+3/2, -z+2 ]

For supplementary data, cf. enclosed CD (file name 'suppl. 184').

## X-ray crystallography data for 184



## X-ray crystallography data for 196



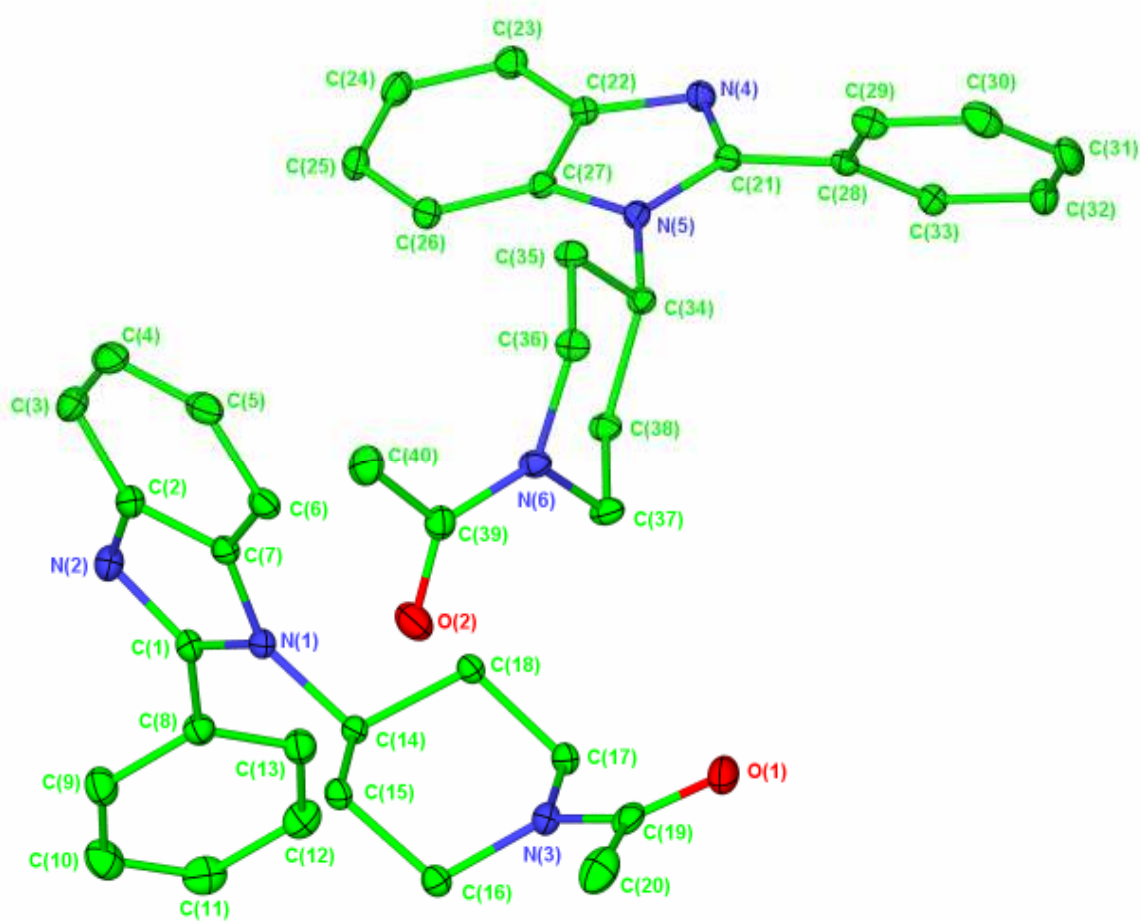
Identification Code	k05bp1
Empirical Formula	C <sub>20</sub> H <sub>21</sub> N <sub>3</sub> O <sub>1</sub>
Formula Weight	321.65
Temperature	150(2) K
Wavelength	0.71073 Å
Crystal System	Monoclinic
Space Group	P2 <sub>1</sub> /n
Unit cell dimensions	a = 13.5250(10) Å $\alpha$ = 90° b = 16.4220(2) Å $\beta$ = 104.561(1)° c = 15.6910(2) Å $\gamma$ = 90°
Crystal Size	0.40 x 0.30 x 0.07 mm

### Notes:

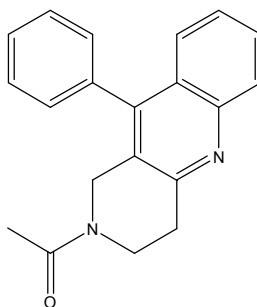
Asymmetric unit consists of 2 molecules, which differ in the relative torsions of the phenyl substituents, and a partial water molecule (O(3)) at 0.25 occupancy. The hydrogen atoms on this water fragment could not be reliably located and hence were omitted from the refinement. Based on N...O and O...O distances, it is highly likely that there is H-bonding associated with this solvent moiety.

For supplementary data, cf. enclosed CD (file name 'suppl. 196).

## X-ray crystallography data for 196



## X-ray crystallography data for 203



Identification Code	h06farm3
Empirical Formula	C <sub>20</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub>
Formula Weight	320.38
Temperature	150(2) K
Wavelength	0.71073 Å
Crystal System	Orthorhombic
Space Group	Pbca
Unit cell dimensions	a = 13.3600(2) Å $\alpha$ = 90° b = 15.2190(3) Å $\beta$ = 90° c = 15.6200(3) Å $\gamma$ = 90°
Crystal Size	0.60 x 0.50 x 0.35 mm

### Notes:

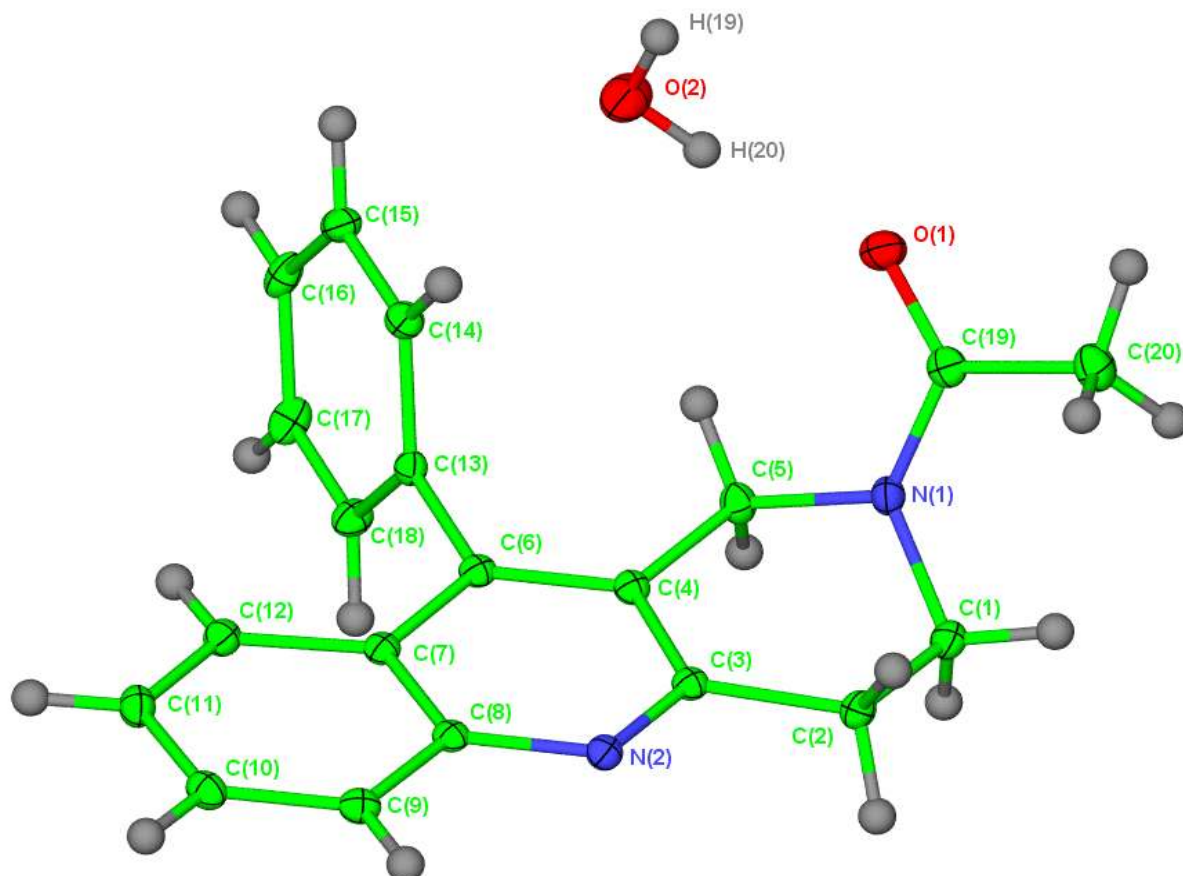
Water hydrogens located and refined at a distance of 0.89 Å from O2. Both of these hydrogen atoms are implicated in hydrogen-bonding.

Hydrogen bonds with  $H \cdots A < r(A) + 2.000 \text{ Å}$  and  $\angle DHA > 110 \text{ deg}$ .

D-H	d(D-H)	d(H...A)	$\angle DHA$	d(D..A)	A
O2-H19	0.890	2.073	172.34	2.958	N2 [ -x+2, y+1/2, -z+1/2 ]
O2-H20	0.890	1.958	174.03	2.845	O1

For supplementary data, cf. enclosed CD (file name 'suppl. 203').

**X-ray crystallography data for 203**





### Appendix III: Compound 184

Table 1. Crystal data and structure refinement for **184**.

Identification code	c:\x-ray\kappa\k07farm3\maxus\k07farm3
Empirical formula	C <sub>24</sub> H <sub>23</sub> Cl N <sub>2</sub> O <sub>2</sub>
Formula weight	406.89
Temperature	150(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	P212121
Unit cell dimensions	a = 8.9500(0) Å $\alpha = 90^\circ$
	b = 10.4450(0) Å $\beta = 90^\circ$
	c = 22.6800(2) Å $\gamma = 90^\circ$
Volume	2120.19(4) Å <sup>3</sup>
Z	4
Density (calculated)	1.275 Mg/m <sup>3</sup>
Absorption coefficient	0.202 mm <sup>-1</sup>
F(000)	856
Crystal size	0.35 x 0.25 x 0.25 mm
Theta range for data collection	3.53 to 30.04°
Index ranges	-12 ≤ h ≤ 12; -14 ≤ k ≤ 14; -31 ≤ l ≤ 30
Reflections collected	42345
Independent reflections	6200 [R(int) = 0.0403]
Reflections observed (>2σ)	5626
Data Completeness	0.996
Absorption correction	None
Refinement method	Full-matrix least-squares on F <sup>2</sup>
Data / restraints / parameters	6200 / 2 / 269
Goodness-of-fit on F <sup>2</sup>	1.038
Final R indices [I>2σ(I)]	R1 = 0.0330 wR2 = 0.0767
R indices (all data)	R1 = 0.0388 wR2 = 0.0799
Absolute structure parameter	-0.01(4)
Largest diff. peak and hole	0.196 and -0.212 eÅ <sup>-3</sup>

**Notes:** H1A and H2 (attached to N1 and N2, respectively) were located and refined at a distance of 0.9 Å from the parent nitrogen atoms.

**Hydrogen bonded polymers in the gross structure:**

**Hydrogen bonds with H...A < r(A) + 2.000 Angstroms and <DHA > 110 deg.**

**D-H      d(D-H)   d(H..A)   <DHA   d(D..A)   A**

N1-H1A      0.906

N2-H2      0.898   1.921   177.51   2.818   O2 [ x+1/2, -y+3/2, -z+2 ]

Table 2. Atomic coordinates ( $\times 10^4$ ) and equivalent isotropic displacement parameters ( $\text{\AA}^2 \times 10^3$ ) for **184**. U(eq) is defined as one third of the trace of the orthogonalized  $U_{ij}$  tensor.

Atom	x	y	z	U(eq)
Cl(1)	13383(1)	5597(1)	6077(1)	41(1)
O(1)	7750(1)	4512(1)	7316(1)	31(1)
O(2)	7183(1)	7843(1)	10380(1)	34(1)
N(1)	8652(1)	4860(1)	8465(1)	26(1)
N(2)	9269(1)	6827(1)	10066(1)	25(1)
C(1)	9448(1)	5227(1)	9001(1)	24(1)
C(2)	10980(1)	4564(1)	8993(1)	28(1)
C(3)	11963(1)	4983(1)	8495(1)	32(1)
C(4)	12551(2)	4214(2)	8099(1)	46(1)
C(5)	8615(1)	4829(1)	9556(1)	26(1)
C(6)	7902(2)	3645(1)	9574(1)	38(1)
C(7)	7205(2)	3208(1)	10081(1)	44(1)
C(8)	7246(2)	3947(1)	10589(1)	38(1)
C(9)	7942(1)	5124(1)	10583(1)	30(1)
C(10)	8615(1)	5580(1)	10067(1)	25(1)
C(11)	8502(1)	7886(1)	10220(1)	25(1)
C(12)	9338(1)	9136(1)	10187(1)	29(1)
C(13)	7475(1)	5594(1)	8248(1)	25(1)
C(14)	6683(2)	6478(1)	8588(1)	29(1)
C(15)	5513(2)	7186(1)	8344(1)	35(1)
C(16)	5100(2)	7018(1)	7760(1)	40(1)
C(17)	5869(2)	6132(1)	7417(1)	36(1)
C(18)	7029(1)	5444(1)	7660(1)	27(1)
C(19)	9075(1)	4849(1)	7042(1)	26(1)
C(20)	9785(2)	6019(1)	7115(1)	28(1)
C(21)	11124(2)	6256(1)	6818(1)	30(1)
C(22)	11713(2)	5321(1)	6456(1)	31(1)
C(23)	11008(2)	4154(1)	6383(1)	34(1)
C(24)	9683(2)	3914(1)	6680(1)	33(1)

Table 3. Bond lengths [ $\text{\AA}$ ] and angles [ $^\circ$ ] for **184**.

Cl(1)-C(22)	1.7476(13)
O(1)-C(19)	1.3848(15)
O(1)-C(18)	1.4035(15)
O(2)-C(11)	1.2362(15)
N(1)-C(13)	1.3922(15)
N(1)-C(1)	1.4616(15)
N(2)-C(11)	1.3475(15)
N(2)-C(10)	1.4285(15)
C(1)-C(5)	1.5195(16)
C(1)-C(2)	1.5361(17)

C(2)-C(3)	1.4980(18)
C(3)-C(4)	1.315(2)
C(5)-C(6)	1.3922(18)
C(5)-C(10)	1.4007(16)
C(6)-C(7)	1.3853(19)
C(7)-C(8)	1.389(2)
C(8)-C(9)	1.3781(19)
C(9)-C(10)	1.3989(16)
C(11)-C(12)	1.5058(17)
C(13)-C(14)	1.3963(17)
C(13)-C(18)	1.4016(16)
C(14)-C(15)	1.3953(19)
C(15)-C(16)	1.387(2)
C(16)-C(17)	1.391(2)
C(17)-C(18)	1.3767(18)
C(19)-C(24)	1.3866(17)
C(19)-C(20)	1.3880(17)
C(20)-C(21)	1.3969(18)
C(21)-C(22)	1.3803(18)
C(22)-C(23)	1.3828(19)
C(23)-C(24)	1.387(2)
C(19)-O(1)-C(18)	117.88(9)
C(13)-N(1)-C(1)	121.22(10)
C(11)-N(2)-C(10)	122.65(10)
N(1)-C(1)-C(5)	112.21(10)
N(1)-C(1)-C(2)	107.89(9)
C(5)-C(1)-C(2)	108.93(10)
C(3)-C(2)-C(1)	113.68(10)
C(4)-C(3)-C(2)	124.94(13)
C(6)-C(5)-C(10)	118.22(11)
C(6)-C(5)-C(1)	119.48(11)
C(10)-C(5)-C(1)	122.18(10)
C(7)-C(6)-C(5)	121.54(12)
C(6)-C(7)-C(8)	119.67(13)
C(9)-C(8)-C(7)	119.91(13)
C(8)-C(9)-C(10)	120.45(12)
C(9)-C(10)-C(5)	120.16(11)
C(9)-C(10)-N(2)	119.26(10)
C(5)-C(10)-N(2)	120.56(10)
O(2)-C(11)-N(2)	122.19(11)
O(2)-C(11)-C(12)	121.33(11)
N(2)-C(11)-C(12)	116.48(10)
N(1)-C(13)-C(14)	123.63(11)
N(1)-C(13)-C(18)	119.35(11)
C(14)-C(13)-C(18)	117.02(11)
C(15)-C(14)-C(13)	120.89(12)
C(16)-C(15)-C(14)	120.71(13)

C(15)-C(16)-C(17)	119.10(13)
C(18)-C(17)-C(16)	119.82(13)
C(17)-C(18)-C(13)	122.45(12)
C(17)-C(18)-O(1)	119.16(11)
C(13)-C(18)-O(1)	118.32(11)
O(1)-C(19)-C(24)	115.05(11)
O(1)-C(19)-C(20)	124.18(11)
C(24)-C(19)-C(20)	120.76(12)
C(19)-C(20)-C(21)	119.42(12)
C(22)-C(21)-C(20)	119.27(12)
C(21)-C(22)-C(23)	121.39(13)
C(21)-C(22)-Cl(1)	120.13(11)
C(23)-C(22)-Cl(1)	118.48(10)
C(22)-C(23)-C(24)	119.44(12)
C(19)-C(24)-C(23)	119.71(12)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ( $\text{\AA}^2 \times 10^3$ ) for **184**. The anisotropic displacement factor exponent takes the form:  $-2 \text{ gpi}^2 [h^2 a^{*2} U_{11} + \dots + 2 h k a^* b^* U_{12}]$

Atom	U11	U22	U33	U23	U13	U12
Cl(1)	33(1)	52(1)	38(1)	7(1)	5(1)	8(1)
O(1)	37(1)	30(1)	27(1)	-4(1)	2(1)	-8(1)
O(2)	21(1)	35(1)	48(1)	-7(1)	1(1)	1(1)
N(1)	29(1)	26(1)	22(1)	-2(1)	1(1)	2(1)
N(2)	21(1)	28(1)	26(1)	-4(1)	1(1)	0(1)
C(1)	31(1)	22(1)	20(1)	0(1)	1(1)	-2(1)
C(2)	31(1)	25(1)	27(1)	2(1)	-2(1)	1(1)
C(3)	29(1)	30(1)	36(1)	2(1)	2(1)	0(1)
C(4)	46(1)	45(1)	48(1)	-4(1)	16(1)	0(1)
C(5)	33(1)	24(1)	22(1)	1(1)	2(1)	-1(1)
C(6)	60(1)	27(1)	27(1)	-2(1)	9(1)	-9(1)
C(7)	69(1)	31(1)	33(1)	1(1)	14(1)	-14(1)
C(8)	53(1)	36(1)	26(1)	5(1)	10(1)	-4(1)
C(9)	33(1)	34(1)	23(1)	-1(1)	1(1)	2(1)
C(10)	24(1)	26(1)	24(1)	0(1)	-1(1)	1(1)
C(11)	23(1)	29(1)	24(1)	-3(1)	-4(1)	2(1)
C(12)	27(1)	28(1)	32(1)	-3(1)	-1(1)	0(1)
C(13)	25(1)	24(1)	26(1)	3(1)	2(1)	-4(1)
C(14)	29(1)	29(1)	30(1)	0(1)	6(1)	-3(1)
C(15)	29(1)	31(1)	46(1)	3(1)	8(1)	2(1)
C(16)	27(1)	41(1)	53(1)	11(1)	-3(1)	2(1)
C(17)	33(1)	42(1)	35(1)	6(1)	-7(1)	-6(1)
C(18)	28(1)	27(1)	27(1)	1(1)	1(1)	-5(1)
C(19)	31(1)	29(1)	19(1)	1(1)	-3(1)	0(1)

C(20)	34(1)	27(1)	24(1)	-2(1)	0(1)	0(1)
C(21)	34(1)	29(1)	28(1)	1(1)	-1(1)	-1(1)
C(22)	29(1)	38(1)	26(1)	6(1)	0(1)	7(1)
C(23)	41(1)	31(1)	30(1)	-3(1)	-1(1)	9(1)
C(24)	42(1)	27(1)	29(1)	-2(1)	-4(1)	1(1)

Table 5. Hydrogen coordinates ( $\times 10^4$ ) and isotropic displacement parameters ( $\text{\AA}^2 \times 10^3$ ) for **184**.

Atom	x	y	z	U(eq)
H(1)	9594	6176	9004	29
H(2A)	11497	4742	9370	33
H(2B)	10827	3628	8966	33
H(3)	12178	5871	8462	38
H(4A)	12361	3320	8116	55
H(4B)	13165	4553	7795	55
H(6)	7893	3125	9230	46
H(7)	6703	2407	10080	53
H(8)	6793	3642	10941	46
H(9)	7966	5628	10931	36
H(12A)	8788	9735	9935	43
H(12B)	10333	8987	10020	43
H(12C)	9435	9497	10584	43
H(14)	6943	6600	8990	35
H(15)	4993	7789	8582	43
H(16)	4303	7501	7596	48
H(17)	5595	6001	7017	44
H(20)	9364	6654	7364	34
H(21)	11625	7051	6865	36
H(23)	11427	3521	6131	41
H(24)	9194	3112	6636	39
H(1A)	9244	4501	8186	38(4)
H(2)	10206(8)	6913(15)	9931(7)	38(4)

# Appendix III: Compound **196**

Table 1. Crystal data and structure refinement for **196**.

Identification code	k05bp1
Empirical formula	C <sub>20</sub> H <sub>21.25</sub> N <sub>3</sub> O <sub>1.13</sub>
Formula weight	321.65
Temperature	150(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P2 <sub>1</sub> /n
Unit cell dimensions	a = 13.5250(10) Å $\angle$ = 90°
	b = 16.4220(2) Å $\angle$ = 104.561(1)°
	c = 15.6910(2) Å $\angle$ = 90°
Volume	3373.15(6) Å <sup>3</sup>
Z	8
Density (calculated)	1.267 Mg/m <sup>3</sup>
Absorption coefficient	0.080 mm <sup>-1</sup>
F(000)	1370
Crystal size	0.40 x 0.30 x 0.07 mm
Theta range for data collection	3.64 to 27.52°
Index ranges	-17 ≤ h ≤ 17; -21 ≤ k ≤ 18; -20 ≤ l ≤ 20
Reflections collected	57457
Independent reflections	7735 [R(int) = 0.0571]
Reflections observed (>2σ)	6290
Data Completeness	0.995
Absorption correction	none
Refinement method	Full-matrix least-squares on F <sup>2</sup>
Data / restraints / parameters	7735 / 0 / 445
Goodness-of-fit on F <sup>2</sup>	1.079
Final R indices [I>2σ(I)]	R <sup>1</sup> = 0.0504 wR <sub>2</sub> = 0.0951
R indices (all data)	R <sup>1</sup> = 0.0678 wR <sub>2</sub> = 0.1014
Largest diff. peak and hole	0.227 and -0.238 eÅ <sup>-3</sup>

**Notes:** Asymmetric unit consists of 2 molecules, which differ in the relative torsions of the phenyl substituents, and a partial water molecule (O(3)) at 0.25 occupancy. The hydrogen atoms on this water fragment could not be reliably located and hence were omitted from the refinement. Based on N...O and O...O distances, it is highly likely that there is h-bonding associated with this solvent moiety.

Table 2. Atomic coordinates ( $\times 10^4$ ) and equivalent isotropic displacement parameters ( $\text{\AA}^2 \times 10^3$ ) for **196**. U(eq) is defined as one third of the trace of the orthogonalized  $U_{ij}$  tensor.

Atom	x	y	z	U(eq)
O(1)	8270(1)	2583(1)	614(1)	46(1)
O(2)	5844(1)	3934(1)	-1422(1)	45(1)
N(1)	4032(1)	3656(1)	963(1)	27(1)
N(2)	2387(1)	3816(1)	1006(1)	37(1)
N(3)	7190(1)	3447(1)	1050(1)	30(1)
N(4)	4032(1)	-756(1)	-1179(1)	26(1)
N(5)	4575(1)	455(1)	-1565(1)	22(1)
N(6)	5822(1)	2707(1)	-2075(1)	27(1)
C(1)	3345(1)	3763(1)	1471(1)	30(1)
C(2)	2438(1)	3732(1)	139(1)	35(1)
C(3)	1644(1)	3728(1)	-633(1)	44(1)
C(4)	1893(1)	3634(1)	-1427(1)	46(1)
C(5)	2910(1)	3546(1)	-1463(1)	40(1)
C(6)	3709(1)	3537(1)	-713(1)	33(1)
C(7)	3454(1)	3632(1)	93(1)	30(1)
C(8)	3626(1)	3799(1)	2442(1)	31(1)
C(9)	3196(1)	4410(1)	2849(1)	38(1)
C(10)	3382(1)	4444(1)	3756(1)	45(1)
C(11)	3992(1)	3861(1)	4268(1)	44(1)
C(12)	4421(1)	3249(1)	3875(1)	39(1)
C(13)	4245(1)	3218(1)	2965(1)	34(1)
C(14)	5156(1)	3639(1)	1276(1)	25(1)
C(15)	5657(1)	4308(1)	855(1)	27(1)
C(16)	6815(1)	4262(1)	1186(1)	30(1)
C(17)	6742(1)	2795(1)	1464(1)	31(1)
C(18)	5583(1)	2803(1)	1140(1)	28(1)
C(19)	7917(1)	3276(1)	622(1)	36(1)
C(20)	8270(1)	3956(1)	125(1)	49(1)
C(21)	4757(1)	-367(1)	-1436(1)	23(1)
C(22)	3316(1)	-161(1)	-1141(1)	24(1)
C(23)	2382(1)	-241(1)	-924(1)	31(1)
C(24)	1790(1)	449(1)	-952(1)	33(1)
C(25)	2120(1)	1206(1)	-1182(1)	31(1)
C(26)	3046(1)	1301(1)	-1393(1)	28(1)
C(27)	3638(1)	601(1)	-1378(1)	23(1)
C(28)	5659(1)	-781(1)	-1602(1)	24(1)
C(29)	5871(1)	-757(1)	-2426(1)	32(1)
C(30)	6679(1)	-1204(1)	-2575(1)	41(1)
C(31)	7276(1)	-1671(1)	-1910(1)	42(1)
C(32)	7078(1)	-1691(1)	-1091(1)	38(1)
C(33)	6269(1)	-1250(1)	-937(1)	29(1)
C(34)	5284(1)	1065(1)	-1762(1)	23(1)

C(35)	4815(1)	1492(1)	-2632(1)	27(1)
C(36)	5535(1)	2143(1)	-2820(1)	29(1)
C(37)	6334(1)	2307(1)	-1248(1)	30(1)
C(38)	5632(1)	1670(1)	-1008(1)	27(1)
C(39)	5589(1)	3508(1)	-2088(1)	31(1)
C(40)	5012(1)	3873(1)	-2952(1)	44(1)
O(3)	541(4)	4642(4)	842(3)	58(1)

Table 3. Bond lengths [ $\text{\AA}$ ] and angles [ $^\circ$ ] for **196**.

O(1)-C(19)	1.2344(18)	O(2)-C(39)	1.2324(18)
N(1)-C(1)	1.3779(18)	N(1)-C(7)	1.3928(17)
N(1)-C(14)	1.4757(17)	N(2)-C(1)	1.3206(18)
N(2)-C(2)	1.386(2)	N(3)-C(19)	1.3528(19)
N(3)-C(17)	1.4613(18)	N(3)-C(16)	1.4652(17)
N(4)-C(21)	1.3165(16)	N(4)-C(22)	1.3882(17)
N(5)-C(21)	1.3778(16)	N(5)-C(27)	1.3913(16)
N(5)-C(34)	1.4719(16)	N(6)-C(39)	1.3517(18)
N(6)-C(37)	1.4632(17)	N(6)-C(36)	1.4651(17)
C(1)-C(8)	1.476(2)	C(2)-C(3)	1.402(2)
C(2)-C(7)	1.403(2)	C(3)-C(4)	1.379(3)
C(4)-C(5)	1.398(2)	C(5)-C(6)	1.383(2)
C(6)-C(7)	1.401(2)	C(8)-C(9)	1.392(2)
C(8)-C(13)	1.393(2)	C(9)-C(10)	1.383(2)
C(10)-C(11)	1.383(2)	C(11)-C(12)	1.381(2)
C(12)-C(13)	1.388(2)	C(14)-C(15)	1.5260(18)
C(14)-C(18)	1.5262(17)	C(15)-C(16)	1.5235(19)
C(17)-C(18)	1.5217(19)	C(19)-C(20)	1.508(2)
C(21)-C(28)	1.4763(18)	C(22)-C(23)	1.3950(18)
C(22)-C(27)	1.4043(18)	C(23)-C(24)	1.382(2)
C(24)-C(25)	1.399(2)	C(25)-C(26)	1.384(2)
C(26)-C(27)	1.3979(18)	C(28)-C(33)	1.3886(19)
C(28)-C(29)	1.3929(19)	C(29)-C(30)	1.385(2)
C(30)-C(31)	1.380(2)	C(31)-C(32)	1.378(2)
C(32)-C(33)	1.383(2)	C(34)-C(35)	1.5247(18)
C(34)-C(38)	1.5267(18)	C(35)-C(36)	1.5228(18)
C(37)-C(38)	1.5227(18)	C(39)-C(40)	1.508(2)
C(1)-N(1)-C(7)	106.04(11)	C(1)-N(1)-C(14)	126.70(11)
C(7)-N(1)-C(14)	127.07(11)	C(1)-N(2)-C(2)	104.59(12)
C(19)-N(3)-C(17)	120.18(12)	C(19)-N(3)-C(16)	125.73(13)
C(17)-N(3)-C(16)	114.01(11)	C(21)-N(4)-C(22)	104.62(11)
C(21)-N(5)-C(27)	106.03(10)	C(21)-N(5)-C(34)	126.48(10)
C(27)-N(5)-C(34)	127.16(10)	C(39)-N(6)-C(37)	120.47(12)
C(39)-N(6)-C(36)	126.10(12)	C(37)-N(6)-C(36)	113.24(11)
N(2)-C(1)-N(1)	113.52(13)	N(2)-C(1)-C(8)	122.03(13)
N(1)-C(1)-C(8)	124.43(12)	N(2)-C(2)-C(3)	129.20(15)
N(2)-C(2)-C(7)	110.64(12)	C(3)-C(2)-C(7)	120.15(15)



C(4)-C(3)-C(2)	118.23(16)	C(3)-C(4)-C(5)	120.94(14)
C(6)-C(5)-C(4)	122.19(16)	C(5)-C(6)-C(7)	116.78(15)
N(1)-C(7)-C(6)	133.10(13)	N(1)-C(7)-C(2)	105.20(13)
C(6)-C(7)-C(2)	121.70(13)	C(9)-C(8)-C(13)	118.83(14)
C(9)-C(8)-C(1)	118.22(13)	C(13)-C(8)-C(1)	122.80(13)
C(10)-C(9)-C(8)	120.77(15)	C(11)-C(10)-C(9)	119.88(15)
C(12)-C(11)-C(10)	120.08(15)	C(11)-C(12)-C(13)	120.22(15)
C(12)-C(13)-C(8)	120.23(14)	N(1)-C(14)-C(15)	112.06(10)
N(1)-C(14)-C(18)	111.39(10)	C(15)-C(14)-C(18)	111.21(11)
C(16)-C(15)-C(14)	110.31(11)	N(3)-C(16)-C(15)	111.02(11)
N(3)-C(17)-C(18)	110.63(11)	C(17)-C(18)-C(14)	110.80(11)
O(1)-C(19)-N(3)	121.84(15)	O(1)-C(19)-C(20)	120.34(15)
N(3)-C(19)-C(20)	117.80(14)	N(4)-C(21)-N(5)	113.65(11)
N(4)-C(21)-C(28)	122.85(11)	N(5)-C(21)-C(28)	123.46(11)
N(4)-C(22)-C(23)	128.94(12)	N(4)-C(22)-C(27)	110.46(11)
C(23)-C(22)-C(27)	120.58(12)	C(24)-C(23)-C(22)	117.92(13)
C(23)-C(24)-C(25)	121.14(13)	C(26)-C(25)-C(24)	121.91(13)
C(25)-C(26)-C(27)	116.90(12)	N(5)-C(27)-C(26)	133.25(12)
N(5)-C(27)-C(22)	105.22(11)	C(26)-C(27)-C(22)	121.53(12)
C(33)-C(28)-C(29)	119.43(13)	C(33)-C(28)-C(21)	118.95(12)
C(29)-C(28)-C(21)	121.48(12)	C(30)-C(29)-C(28)	119.79(14)
C(31)-C(30)-C(29)	120.19(14)	C(32)-C(31)-C(30)	120.33(14)
C(31)-C(32)-C(33)	119.87(15)	C(32)-C(33)-C(28)	120.39(14)
N(5)-C(34)-C(35)	110.83(10)	N(5)-C(34)-C(38)	111.70(10)
C(35)-C(34)-C(38)	111.85(11)	C(36)-C(35)-C(34)	110.74(11)
N(6)-C(36)-C(35)	110.13(11)	N(6)-C(37)-C(38)	110.06(11)
C(37)-C(38)-C(34)	110.22(11)	O(2)-C(39)-N(6)	121.63(13)
O(2)-C(39)-C(40)	120.59(13)	N(6)-C(39)-C(40)	117.78(13)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ( $\text{\AA}^2 \times 10^3$ ) for **196**. The anisotropic displacement factor exponent takes the form:  $-2 \text{ gpi}^2 [h^2 a^{*2} U11 + \dots + 2 h k a^* b^* U$

Atom	U11	U22	U33	U23	U13	U12
O(1)	36(1)	53(1)	44(1)	-16(1)	0(1)	14(1)
O(2)	55(1)	32(1)	45(1)	-7(1)	9(1)	2(1)
N(1)	26(1)	23(1)	28(1)	-2(1)	1(1)	3(1)
N(2)	29(1)	33(1)	45(1)	-2(1)	3(1)	6(1)
N(3)	26(1)	28(1)	34(1)	0(1)	2(1)	4(1)
N(4)	26(1)	23(1)	31(1)	0(1)	8(1)	-2(1)
N(5)	21(1)	21(1)	24(1)	2(1)	5(1)	-1(1)
N(6)	28(1)	23(1)	26(1)	2(1)	3(1)	-5(1)
C(1)	29(1)	23(1)	37(1)	-2(1)	6(1)	4(1)
C(2)	31(1)	26(1)	44(1)	-2(1)	-1(1)	4(1)
C(3)	33(1)	36(1)	53(1)	-2(1)	-9(1)	5(1)
C(4)	46(1)	34(1)	42(1)	-3(1)	-17(1)	2(1)
C(5)	53(1)	27(1)	32(1)	-3(1)	-4(1)	0(1)
C(6)	40(1)	22(1)	32(1)	-2(1)	0(1)	2(1)
C(7)	32(1)	20(1)	31(1)	-1(1)	-4(1)	2(1)
C(8)	27(1)	29(1)	36(1)	-3(1)	9(1)	-4(1)
C(9)	38(1)	35(1)	45(1)	-3(1)	15(1)	3(1)
C(10)	47(1)	44(1)	49(1)	-10(1)	25(1)	-4(1)
C(11)	43(1)	57(1)	35(1)	-5(1)	18(1)	-11(1)
C(12)	35(1)	46(1)	38(1)	6(1)	9(1)	-4(1)
C(13)	32(1)	31(1)	39(1)	0(1)	11(1)	-1(1)
C(14)	25(1)	22(1)	24(1)	-1(1)	0(1)	3(1)
C(15)	30(1)	21(1)	28(1)	1(1)	3(1)	4(1)
C(16)	32(1)	23(1)	33(1)	0(1)	4(1)	1(1)
C(17)	31(1)	22(1)	35(1)	0(1)	0(1)	5(1)
C(18)	29(1)	21(1)	30(1)	-2(1)	1(1)	2(1)
C(19)	26(1)	49(1)	26(1)	-7(1)	-5(1)	4(1)
C(20)	40(1)	72(1)	34(1)	5(1)	9(1)	7(1)
C(21)	24(1)	20(1)	22(1)	-1(1)	3(1)	-1(1)
C(22)	23(1)	24(1)	24(1)	-1(1)	4(1)	-1(1)
C(23)	27(1)	32(1)	34(1)	0(1)	9(1)	-4(1)
C(24)	22(1)	42(1)	35(1)	-2(1)	8(1)	1(1)
C(25)	27(1)	33(1)	31(1)	-2(1)	3(1)	8(1)
C(26)	27(1)	25(1)	27(1)	1(1)	2(1)	3(1)
C(27)	22(1)	25(1)	21(1)	0(1)	2(1)	-1(1)
C(28)	24(1)	18(1)	30(1)	-4(1)	6(1)	-3(1)
C(29)	37(1)	29(1)	31(1)	-3(1)	11(1)	-6(1)
C(30)	47(1)	38(1)	46(1)	-14(1)	28(1)	-11(1)
C(31)	32(1)	32(1)	67(1)	-14(1)	22(1)	0(1)
C(32)	29(1)	29(1)	53(1)	-2(1)	6(1)	4(1)
C(33)	29(1)	25(1)	33(1)	-2(1)	6(1)	0(1)
C(34)	23(1)	21(1)	24(1)	1(1)	5(1)	-3(1)
C(35)	31(1)	26(1)	22(1)	0(1)	3(1)	-5(1)

C(36)	34(1)	28(1)	25(1)	2(1)	7(1)	-4(1)
C(37)	29(1)	28(1)	27(1)	2(1)	-3(1)	-6(1)
C(38)	31(1)	26(1)	22(1)	2(1)	-1(1)	-3(1)
C(39)	27(1)	28(1)	37(1)	2(1)	9(1)	-1(1)
C(40)	44(1)	33(1)	49(1)	10(1)	1(1)	4(1)
O(3)	42(3)	90(4)	44(3)	8(3)	14(2)	11(3)

Table 5. Hydrogen coordinates (  $\times 10^4$  ) and isotropic displacement parameters ( $\text{\AA}^2 \times 10^3$ ) for **196**.

Atom	x	y	z	U(eq)
H(3)	953	3787	-610	53
H(4)	1367	3630	-1958	55
H(5)	3058	3489	-2021	48
H(6)	4397	3471	-743	39
H(9)	2769	4808	2499	46
H(10)	3090	4867	4027	54
H(11)	4117	3881	4891	52
H(12)	4838	2847	4229	47
H(13)	4547	2798	2698	40
H(14)	5332	3745	1924	30
H(15A)	5459	4245	206	33
H(15B)	5416	4847	1000	33
H(16A)	7132	4668	870	36
H(16B)	7017	4396	1822	36
H(17A)	6941	2866	2112	37
H(17B)	7008	2263	1325	37
H(18A)	5290	2388	1464	34
H(18B)	5382	2661	506	34
H(20A)	8791	3751	-155	73
H(20B)	8559	4396	534	73
H(20C)	7688	4165	-328	73
H(23)	2160	-754	-762	37
H(24)	1147	408	-812	40
H(25)	1695	1669	-1193	37
H(26)	3270	1818	-1542	33
H(29)	5463	-434	-2884	38
H(30)	6823	-1190	-3137	49
H(31)	7826	-1980	-2017	50
H(32)	7496	-2007	-632	45
H(33)	6129	-1268	-374	35
H(34)	5904	767	-1832	28
H(35A)	4673	1088	-3116	32
H(35B)	4160	1747	-2607	32
H(36A)	6155	1882	-2921	35
H(36B)	5195	2447	-3359	35
H(37A)	6518	2718	-773	36

H(37B)	6970	2044	-1312	36
H(38A)	5029	1940	-883	33
H(38B)	5999	1377	-470	33
H(40A)	4819	4434	-2857	66
H(40B)	4395	3551	-3195	66
H(40C)	5446	3869	-3367	66

# Appendix III: Compound **203**

Table 1. Crystal data and structure refinement for **203**.

Identification code	h06farm3
Empirical formula	C <sub>20</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub>
Formula weight	320.38
Temperature	150(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	Pbca
Unit cell dimensions	a = 13.3600(2) Å $\alpha = 90^\circ$
	b = 15.2190(3) Å $\beta = 90^\circ$
	c = 15.6200(3) Å $\gamma = 90^\circ$
Volume	3175.95(10) Å <sup>3</sup>
Z	8
Density (calculated)	1.340 Mg/m <sup>3</sup>
Absorption coefficient	0.087 mm <sup>-1</sup>
F(000)	1360
Crystal size	0.60 x 0.50 x 0.35 mm
Theta range for data collection	4.01 to 27.50°
Index ranges	-17 ≤ h ≤ 17; -19 ≤ k ≤ 19; -20 ≤ l ≤ 20
Reflections collected	52442
Independent reflections	3638 [R(int) = 0.0784]
Reflections observed (>2σ)	2467
Data Completeness	0.996
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.97 and 0.92
Refinement method	Full-matrix least-squares on F <sup>2</sup>
Data / restraints / parameters	3638 / 2 / 227
Goodness-of-fit on F <sup>2</sup>	1.050
Final R indices [I>2σ(I)]	R <sup>1</sup> = 0.0430 wR <sub>2</sub> = 0.1009
R indices (all data)	R <sup>1</sup> = 0.0783 wR <sub>2</sub> = 0.1144
Largest diff. peak and hole	0.265 and -0.225 eÅ <sup>-3</sup>

**Notes:** Water hydrogens located and refined at a distance of 0.89 Å from O2. Both of these hydrogen atoms are implicated in hydrogen-bonding.

**Hydrogen bonds with H..A < r(A) + 2.000 Angstroms and <DHA > 110 deg.**

D-H	d(D-H)	d(H..A)	<DHA	d(D..A)	A
O2-H19	0.890	2.073	172.34	2.958	N2 [ -x+2, y+1/2, -z+1/2 ]
O2-H20	0.890	1.958	174.03	2.845	O1

Table 2. Atomic coordinates ( $\times 10^4$ ) and equivalent isotropic displacement parameters ( $\text{\AA}^2 \times 10^3$ ) for **203**. U(eq) is defined as one third of the trace of the orthogonalized  $U_{ij}$  tensor.

Atom	x	y	z	U(eq)
O(1)	12094(1)	3224(1)	1942(1)	36(1)
O(2)	10227(1)	4114(1)	1812(1)	53(1)
N(1)	12254(1)	1786(1)	1611(1)	26(1)
N(2)	9867(1)	123(1)	1572(1)	23(1)
C(1)	12504(1)	871(1)	1799(1)	27(1)
C(2)	11563(1)	362(1)	2020(1)	26(1)
C(3)	10681(1)	598(1)	1458(1)	22(1)
C(4)	10736(1)	1310(1)	868(1)	22(1)
C(5)	11672(1)	1872(1)	825(1)	27(1)
C(6)	9920(1)	1481(1)	353(1)	21(1)
C(7)	9048(1)	953(1)	434(1)	22(1)
C(8)	9045(1)	291(1)	1072(1)	21(1)
C(9)	8162(1)	-202(1)	1219(1)	26(1)
C(10)	7323(1)	-45(1)	745(1)	28(1)
C(11)	7329(1)	598(1)	94(1)	29(1)
C(12)	8167(1)	1083(1)	-59(1)	26(1)
C(13)	9927(1)	2237(1)	-259(1)	22(1)
C(14)	9726(1)	3080(1)	38(1)	27(1)
C(15)	9720(1)	3782(1)	-527(1)	30(1)
C(16)	9909(1)	3651(1)	-1388(1)	31(1)
C(17)	10111(1)	2818(1)	-1686(1)	32(1)
C(18)	10115(1)	2109(1)	-1127(1)	28(1)
C(19)	12382(1)	2479(1)	2137(1)	28(1)
C(20)	12890(1)	2326(1)	2987(1)	39(1)

Table 3. Bond lengths [ $\text{\AA}$ ] and angles [ $^\circ$ ] for **203**.

O(1)-C(19)	1.2364(18)	N(1)-C(19)	1.3473(19)
N(1)-C(5)	1.4587(18)	N(1)-C(1)	1.4618(19)
N(2)-C(3)	1.3179(18)	N(2)-C(8)	1.3713(18)
C(1)-C(2)	1.517(2)	C(2)-C(3)	1.5123(19)
C(3)-C(4)	1.425(2)	C(4)-C(6)	1.379(2)
C(4)-C(5)	1.517(2)	C(6)-C(7)	1.421(2)
C(6)-C(13)	1.495(2)	C(7)-C(8)	1.4175(19)
C(7)-C(12)	1.419(2)	C(8)-C(9)	1.4168(19)
C(9)-C(10)	1.364(2)	C(10)-C(11)	1.410(2)
C(11)-C(12)	1.362(2)	C(13)-C(14)	1.391(2)
C(13)-C(18)	1.393(2)	C(14)-C(15)	1.386(2)
C(15)-C(16)	1.383(2)	C(16)-C(17)	1.377(2)
C(17)-C(18)	1.388(2)	C(19)-C(20)	1.509(2)
C(19)-N(1)-C(5)	120.72(12)	C(19)-N(1)-C(1)	126.40(12)

C(5)-N(1)-C(1)	112.11(12)	C(3)-N(2)-C(8)	118.83(12)
N(1)-C(1)-C(2)	110.09(12)	C(3)-C(2)-C(1)	113.08(12)
N(2)-C(3)-C(4)	123.11(12)	N(2)-C(3)-C(2)	115.76(12)
C(4)-C(3)-C(2)	121.10(12)	C(6)-C(4)-C(3)	118.73(13)
C(6)-C(4)-C(5)	121.29(12)	C(3)-C(4)-C(5)	119.97(12)
N(1)-C(5)-C(4)	110.60(11)	C(4)-C(6)-C(7)	119.28(13)
C(4)-C(6)-C(13)	120.89(13)	C(7)-C(6)-C(13)	119.76(12)
C(8)-C(7)-C(12)	118.52(13)	C(8)-C(7)-C(6)	117.83(12)
C(12)-C(7)-C(6)	123.58(13)	N(2)-C(8)-C(9)	118.44(12)
N(2)-C(8)-C(7)	122.05(12)	C(9)-C(8)-C(7)	119.50(12)
C(10)-C(9)-C(8)	120.23(13)	C(9)-C(10)-C(11)	120.53(14)
C(12)-C(11)-C(10)	120.42(13)	C(11)-C(12)-C(7)	120.75(14)
C(14)-C(13)-C(18)	119.24(13)	C(14)-C(13)-C(6)	119.72(12)
C(18)-C(13)-C(6)	121.03(13)	C(15)-C(14)-C(13)	119.98(13)
C(16)-C(15)-C(14)	120.54(14)	C(17)-C(16)-C(15)	119.73(14)
C(16)-C(17)-C(18)	120.31(14)	C(17)-C(18)-C(13)	120.20(14)
O(1)-C(19)-N(1)	121.81(14)	O(1)-C(19)-C(20)	119.91(14)
N(1)-C(19)-C(20)	118.28(14)		

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ( $\text{\AA}^2 \times 10^3$ ) for **203**. The anisotropic displacement factor exponent takes the form:  $-2 \text{ gpi}^2 [h^2 a^{*2} U_{11} + \dots + 2 h k a^* b^* U_{12}]$

Atom	U11	U22	U33	U23	U13	U12
O(1)	42(1)	29(1)	37(1)	1(1)	-2(1)	2(1)
O(2)	70(1)	52(1)	36(1)	-15(1)	-13(1)	21(1)
N(1)	24(1)	28(1)	26(1)	2(1)	-5(1)	-1(1)
N(2)	24(1)	23(1)	21(1)	-1(1)	1(1)	0(1)
C(1)	24(1)	29(1)	29(1)	1(1)	-1(1)	3(1)
C(2)	26(1)	27(1)	23(1)	3(1)	-2(1)	2(1)
C(3)	24(1)	23(1)	19(1)	-2(1)	1(1)	2(1)
C(4)	23(1)	23(1)	19(1)	-2(1)	1(1)	0(1)
C(5)	28(1)	31(1)	23(1)	4(1)	-3(1)	-3(1)
C(6)	26(1)	22(1)	17(1)	-2(1)	2(1)	2(1)
C(7)	24(1)	22(1)	18(1)	-4(1)	1(1)	3(1)
C(8)	24(1)	21(1)	19(1)	-4(1)	2(1)	2(1)
C(9)	30(1)	24(1)	24(1)	-1(1)	3(1)	-1(1)
C(10)	25(1)	29(1)	31(1)	-3(1)	2(1)	-4(1)
C(11)	24(1)	35(1)	29(1)	-2(1)	-3(1)	2(1)
C(12)	28(1)	27(1)	22(1)	-1(1)	-1(1)	3(1)
C(13)	20(1)	23(1)	23(1)	1(1)	-1(1)	0(1)
C(14)	29(1)	28(1)	25(1)	-2(1)	-1(1)	0(1)
C(15)	25(1)	23(1)	43(1)	-1(1)	-1(1)	1(1)
C(16)	21(1)	33(1)	39(1)	16(1)	2(1)	2(1)
C(17)	31(1)	40(1)	25(1)	8(1)	5(1)	6(1)

C(18)	31(1)	28(1)	25(1)	0(1)	2(1)	4(1)
C(19)	25(1)	31(1)	28(1)	0(1)	2(1)	-2(1)
C(20)	41(1)	43(1)	31(1)	-4(1)	-8(1)	-1(1)

Table 5. Hydrogen coordinates ( $\times 10^4$ ) and isotropic displacement parameters ( $\text{\AA}^2 \times 10^3$ ) for **203**.

Atom	x	y	z	U(eq)
H(1A)	12978	847	2286	33
H(1B)	12833	602	1295	33
H(2A)	11701	-275	1962	31
H(2B)	11386	477	2625	31
H(5A)	12085	1688	331	33
H(5B)	11482	2495	741	33
H(9)	8154	-644	1648	31
H(10)	6730	-371	854	34
H(11)	6745	693	-239	35
H(12)	8163	1512	-500	31
H(14)	9594	3175	629	32
H(15)	9584	4357	-322	36
H(16)	9900	4134	-1773	37
H(17)	10248	2728	-2276	38
H(18)	10247	1535	-1337	33
H(20A)	12986	2890	3279	58
H(20B)	13542	2048	2893	58
H(20C)	12472	1942	3342	58
H(19)	10160(17)	4379(14)	2317(7)	88(8)
H(20)	10796(10)	3809(15)	1824(18)	103(10)